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The Role of Epithelial Markers in Breast Cancer Metastasis: Systematic Review and Meta-Analysis

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Abstract

Epithelial-mesenchymal transition is believed to be a fundamental component of cancer metastasis. Hence, epithelial markers have emerged as potential therapeutic targets and diagnostic markers of metastatic cancers, leading to their significance in cancer research. In this review, studies on 15 different markers were identified to elucidate further the role of epithelial markers in breast cancer metastasis. Based on the studies, the respective role of the epithelial markers in metastatic breast cancer was derived. The cellular mechanisms guiding the markers' behavior were investigated by identifying and describing their associated miRNAs. The studies of 5 epithelial markers that had identified cellular mechanisms affecting breast cancer metastasis were screened for undergoing meta-analysis. Twenty-one studies in total had sufficient data to undergo meta-analysis. Based on the content of the studies and the conducted meta-analysis, the results' limitations, strengths, and implications were discussed in detail. Although, due to the limited amount of studies, definite conclusions cannot be made, the meta-analysis revealed novel inferences and confirmed inferences made by other researchers on the role of the specific epithelial marker in metastatic breast cancer. Additionally, the study provides insight into significant gaps in the field and urges greater exploration of the topic.

Keywords: Epithelial markers, Breast cancer, EMT, Metastasis, miRNA

1. Introduction

Most instances of morbidity and mortality due to malignant tumors in women are correlated with breast cancer. Most breast cancer-related deaths occur due to metastasis, or the process by which an original primary tumor evolves to a distal secondary tumor (Hagemeister et al., 1980). Metastasis is a highly complex process that requires epithelial-mesenchymal transition (EMT) (Sun et al., 2020). During EMT epithelial cells repress their epithelial characteristics and acquire mesenchymal, as a result of changes in gene expression and gene regulation mechanisms. Since the loss of epithelial markers is associated with EMT, and hence metastatic progression, revealing their status in breast cancer metastasis can potentially reveal new therapeutic targets and biomarkers for diagnosis of metastatic breast cancer (MBC) (Tyler & Tirosh, 2021). The objective of this study is to provide a comprehensive review and meta-analysis on the role of epithelial markers in MBC, and to explore their transcriptional regulation via miRNAs. We hypothesize that specific epithelial markers play a crucial role in MBC and are influenced by distinct miRNAs, which can also serve as potential diagnostic markers and therapeutic targets.

As per the guidelines proposed by the EMT International Association (TEMTIA), we acknowledge the need of a distinct description of the cellular mechanisms guiding the role of the epithelial markers in metastasis and the contribution of genetic alterations, due to the complexity of EMT and its context dependent nature (J. Yang et al., 2020). As a result, all the epithelial markers identified in the review are associated with microRNA(miRNA), a type of transcription regulator. Not only is the inclusion of miRNAs in accordance with the guidelines proposed by

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TEMTIA, but since miRNA dysregulation has been detected in multiple metastatic cancers, including breast cancer, identification of miRNA sequences and their roles can contribute to novel cancer detection techniques through their utilization as biomarkers and components of new treatments through gene editing techniques (J. Yang et al., 2020). It has been shown that certain miRNA changes can be corrected using miRNA mimics or antagomirs, normalizing the signaling pathways and the gene regulatory network, and reversing the phenotype in malignant cells (O'Bryan et al., 2017). Due to the emergence of precise gene-editing techniques such as CRISPR-Cas9, miRNAs have increased potential in cancer treatment and should be a focus of research (Godden et al., 2022). Following the TEMTIA guidelines, a necessary criterion for the markers, whose studies were subjected to meta-analysis, was clear cellular processes through which they influence MBC. Fundamental characteristics acknowledged in the analysis of the results are the molecular (luminal A/B, Triple-negative, and HER2+ enriched) and/or histological breast cancer subtypes of the samples included in the study, their tumor progression stage, and microenvironment (Q. Liu et al., 2017). The epithelial markers selected for the meta-analysis portion of the study due to their defined cellular mechanisms were B-Catenin, Nectin-4, MUC1, JAM-A, and CD44.

B-catenin is a multifunctional membrane protein that's a key component of cell-cell adhesion machinery as an intracellular signal inducer in the Wnt pathway (Shang et al., 2017). The Wnt/B-catenin signaling pathway has been shown to have a regulative role in multiple cell processes including cell motility, making its disruption a causative factor for multiple pathologies, including MBC (Komiya & Habas, 2008). In normal cells the absence of Wnt leads to the phosphorylation of cytoplasmic β-catenin by GSK3β and casein kinase Iα (CK Iα), which in turn prevents nuclear accumulation of β-catenin, allowing its ubiquitination and subsequent degradation by the ubiquitin/proteasome system (Shang et al., 2017). Nevertheless, when Wnt binds to Frizzled (FZD), it activates Disheveled (Dsh), whose activation inhibits GSK3β (Zeng et al., 2008). As a result, B-catenin is not degraded and accumulates in the cytoplasm and nucleus. There, it interacts with transcription coregulators like T cell factor/lymphocyte enhancer factor (Tcf/Lef), forming a B-catenin/Lef/Tcf complex. This complex transactivates the gene that encodes cyclin D1, leading to overgrowth of cells in the lobules and ducts inside the breast (Buechel et al., 2021). Nuclear accumulation of B-catenin also results in the loss of E-cadherin and consequent loss of cell polarity and adhesion, promoting the process of EMT, and therefore metastasis (Buechel et al., 2021).

Nectins are members of the immunoglobulin superfamily (IgSF) and are components of E-cadherin-based adherens junctions in epithelial cells, thereby having a vital role in the enhancement of cellular viability and movement ability (Mandai et al., 2015). Generally, studies agree that Nectin-4 is not expressed in normal epithelium, which contributes to their increased potential to act as a biomarker or treatment in MBC. Nectin-4 has been shown to affect metastasis by modulating the CXCR4/CXCL12-LYVE-1- axis (Sethy et al., 2021). Nectin-4 overexpression leads to an increase in CXCR4 expression and LYVE-1-lymphatic vessel density (LVD). Upregulation of LVD has been associated with increased invasive abilities and poor prognosis in patients (Ramani et al., 2012). CXCR4-expressing cancer cells are attracted by CXCL12- expressing organs, thereby initiating metastasis to distant organs (Guo et al., 2016). Additionally, ADAM-17, whose expression is driven by cancer stem cells, sheds the Nectin-4 ectodomain, which interacts with endothelial Integrin-B4. This interaction promotes metastasis in breast cancer stem cells by activating the Src-PI3K-AKT-iNOS axis (Siddharth et al., 2018). In particular, Nectin-4 has been shown to promote breast cancer stem cell metastasis via the Pi3k/Akt axis through WNT/β-Catenin signaling (Siddharth et al., 2017).

JAM-A is an immunoglobulin-like molecule that acts as a tight junction protein, and as such has a role in tumor cell adhesion, polarity, invasion and migration (Severson & Parkos, 2009). The cellular mechanisms through which JAM-A affects metastasis indicate that JAM-A operates differently in tissue- and cell- specific contexts. For example, by inhibiting the Akt/B-catenin signaling pathway, JAM-A disrupts Akt-mediated phosphorylation of B-catenin, thereby preventing its accumulation in the nucleus and therefore metastasis (Nava et al., 2011). In contrast, in HER2-positive breast cancer, a type of cancer that has increased proliferation ability, increased JAM-A expression promotes HER2 expression by causing the binding of FOXA1 to the HER2 gene promoter (Cruz et al., 2022). HER2 has also been shown to activate the PI3K/Akt pathway, where PI3K phosphorylation leads to Akt2 phosphorylation, whose amplification has been associated with MBC (Milella et al., 2015). JAM-A has also been shown to activate Rap1 GTPase and β1-integrin, both of which lead to increased metastatic potential of breast tumors. Rap1 activation prohibits metastasis in other types of cancers (Yi-Lei et al., 2017).

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MUC-1 is a transmembrane membrane glycoprotein associated with the protection of the epithelial layer by providing lubrication of luminal epithelial surfaces, thereby promoting motility (W. Chen et al., 2021). By interacting with ICAM-1, an adhesion receptor, glycosylated MUC-1, facilitates the interaction between epithelial and endothelial cells. This process enables adhesion of circulating cancer cells to the inner lining of the blood vessel, directly or as a result of a precedent interaction with E–selectin (Hayashi et al., 2001). Glycosylated MUC1 also interacts with Src, a non-receptor tyrosine kinases that says a key role in signal transduction pathways, thereby inducing pro-migratory Rac1- and Cdc42-dependent actin reorganization at sites of contact with endothelial cells, which promotes an invasive phenotype in the tumor cell (Shen et al., 2008). MUC1 can also drive tumor angiogenesis by upregulating vascular-endothelial growth factor (VEGF), thereby promoting endothelial migration and tube formation (Khodabakhsh et al., 2021). Epidermal growth factor receptor (EGFR) stimulates growth of cancer cells, and activates STAT1 and STAT3 in breast cancer, which promote cell survival and motility. MUC1 and EGFR have a positive feedback relationship in breast cancer, resulting in dependence of EGFR prolongation on MUC1. Hence, STAT3 induces the expression of Twist one, which forms a complex with MUC-1 that results in its expression in an auto-indicative loop, accounting for its upregulation in breast cancer (Bitler et al., 2010). A subunit of MUC-1, MUC1-C, can also induce EMT and thus metastasis by activating the inflammatory NF-κB p65 pathway, which induces the transcription of ZEB1 and B-cell lymphoma 2-related protein A1 (BCL2A1) (Ahmad et al., 2009).

CD44 is a cell-surface glycoprotein involved in cell-cell interactions, adhesion, and motility, and CD44 has been used as a surface marker for breast cancer stem cells (CSCs) (Thapa & Wilson, 2016). Breast CSCs that exhibit CD44+/CD24- are potentially one of the main factors contributing to relapse of triple negative breast cancer (TNBC) due to their exacerbated self-renewal and differentiation abilities (X. Qiao et al., 2021). CD44 expression activates Rho GTPases and PI3K/AKT and MAPK-Ras, thereby promoting cytoskeletal remodeling and invasion. CD44 promotes cleavage of hyaluronan, resulting in modifications of the tumor microenvironment and essentially tumor progression. Expression of CD44 promotes docking of collagen specific MMP9. When MMP9 is found in the edges of migratory cells it promotes collagen degradation, thereby leading to an invasive phenotype, and cleavage of TGFB which also promotes invasion (Louderbough & Schroeder, 2011). Under specific conditions the ECM component hyaluronate stimulates CD44 to bind with merlin, a tumor suppressor protein, thereby conferring growth arrest in tumor cells (Herrlich et al., 2006). Other ways through which CD44 can prevent metastasis is by activating caspase-3 and hence promoting apoptosis of tumor cells, or by inhibiting PI3K activation/AKT phosphorylation (Ghatak et al., 2002).

The present meta-analysis distinguishes itself by integrating all studied epithelial markers linked to MBC and discussing their transcriptional regulation via miRNAs. We anticipated to encounter a correlation between epithelial markers and breast cancer metastasis, as well as miRNAs influence. Both miRNAs and markers could be used as therapeutic markers for targeted therapy. This approach results in a uniquely structured review, offering a more detailed depiction of the process than previously seen in other systematic reviews on the subject.

2. Materials and Methods

Marker selection: All known epithelial cell markers were identified via the Bio-techne database (Epithelial Cell Markers and Intracellular Molecules, n.d.), and were assessed for role in cell adhesion through the National Library of Medicine database. To identify which of the remaining molecules are the subject of breast cancer studies, advanced search was performed via marker name, boolean operator “and”, and “breast cancer metastasis”. Review articles were identified through keywords: epithelial-mesenchymal transition (EMT), tumor marker, cancer metastasis, name of epithelial cell marker and were manually searched for more references on the topic. Markers implicated in more than five breast cancer metastasis studies proceeded to the next selection stage. These markers were then examined for studies exploring their connection with miRNAs. Table 1 presents the markers that play a role in MBC, their associated miRNAs, and studies discussing their involvement in MBC. The studies of markers that had identified cellular mechanisms in the context of MBC and associated miRNAs, were discussed in detail and analyzed for meta-analysis eligibility.

Meta-analysis: Odds ratio (OR) was used to examine the association between the expression of epithelial markers

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and their prevalence in MBC. OR represents the likelihood of an event occurring when exposed to a specific factor, in contrast to the likelihood of the event without that exposure. In the current context, the OR offers insight into the likelihood of the occurrence of MBC in the presence of an epithelial marker, in contrast to the likelihood of MBC without the epithelial marker. A 2x2 contingency table was set up, with one axis indicating the presence or absence of MBC and the other indicating the expression or non-expression of the epithelial marker. The calculated OR was derived using the formula: [OR = $\frac{ad}{bc}$], where 'a' denotes individuals with both the epithelial marker and MBC, 'b' signifies those with the marker but without MBC, 'c' represents those without the marker but with MBC, and 'd' identifies those without either the marker or MBC. In order for studies to be eligible for the meta-analysis, they should have reported their results such that marker transcription acts as a dependent variable, and variables measuring metastasis act as independent variables. +/- metastasis or +/- lymph node involvement would be seen as variables measuring metastasis. An OR value of 1 would suggest no association between the epithelial marker and MBC. In contrast, an OR greater than 1 would indicate an increased likelihood of MBC in the presence of the marker, whereas an OR less than 1 would suggest a decreased likelihood. The 95% confidence interval (CI) provides an estimation of the accuracy of the OR. A wide range of CI suggests that the OR's accuracy is low, while a narrow CI suggests greater accuracy. The 95% CI doesn't reflect statistical significance in the same manner as the p-value. However, if the 95% CI doesn't cross the null value (e.g., OR=1), it's often interpreted as evidence of statistical significance. Heterogeneity refers to the variability or differences in study outcomes. Analyzing heterogeneity offers insights into the influence of varying methodologies and conditions on the study outcomes. The heterogeneity of the data was assessed using tau square and chi square to make sure that all studies are evaluating the same effect. The tau squared (Tau^2) represents between-study variance, with elevated values indicating substantial inter-study variability. The chi-square (Chi^2) tests the hypothesis that the studies are evaluating the same effect, with a low p-value (typically < 0.05) suggesting that heterogeneity is present beyond chance. The p-value represents the probability of observing the given data, or more extreme data, under the null hypothesis of no effect. A p-value less than 0.05 is conventionally deemed indicative of statistical significance. Furthermore, the I^2 statistic provides a quantification of the proportion of total variation across studies that's attributable to heterogeneity rather than chance. In the context of this research, an I^2 value below 50% was interpreted as indicating satisfactory homogeneity. All statistical analyses were conducted using RevMan Version 5.

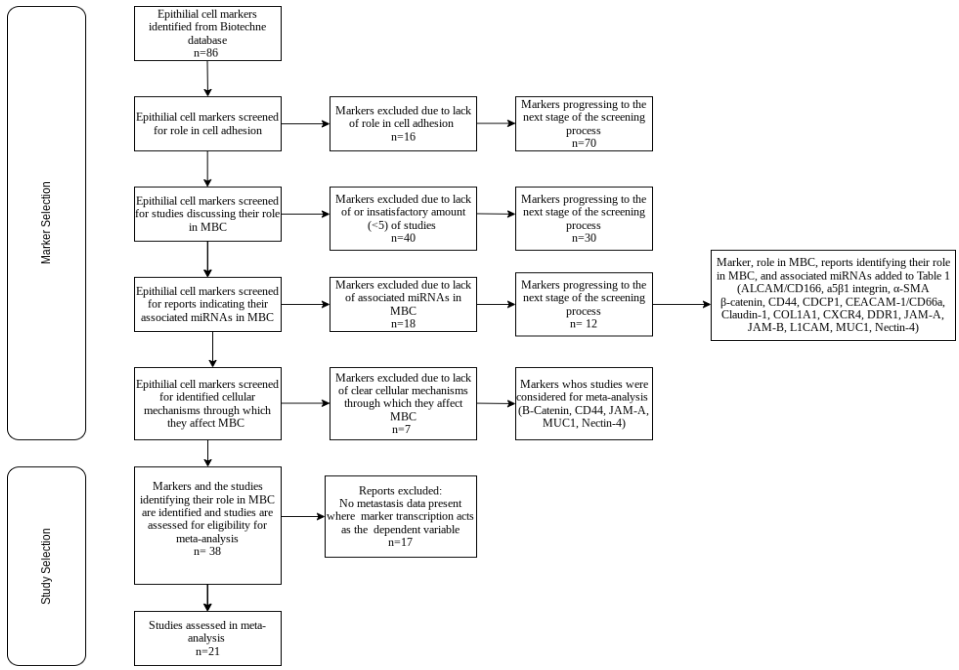

Figure 1. Flow chart representing selection procedure for epithelial cell markers and study selection for meta-analysis based on studies listed in Table 1.

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3. Results

12 epithelial markers were shown to have a relationship with MBC in more than 5 studies, and an association with a type of miRNA, which is illustrated in Table 1. The relationship between miRNAs and metastasis was portrayed in Figure 2 and forest plots were produced to depict the statistical analysis undergone by the eligible studies of the 5 selected markers.

Table 1 Epithelial markers associated with MBC, their role in MBC, the studies discussing their role in MBC, and their associated miRNAs

Name of Marker	Role of Marker in Metastasis	Associated miRNAs	Studies discussing role of marker in metastasis
ALCAM/CD166	tumor suppressor	miR-125	(Davies et al., 2008), (Akamn et al., 2015)
α-SMA	oncoprotein	miR-200c	(Tang et al., 2015), (Mierke et al., 2011)
Integrin α5β1	oncoprotein	miR-31,-149	(Wang, Yanfang, et al. 2011), (Chan, S. 2014) , (Augoff, K., et al 2011)
β-catenin	oncoprotein	miR-200c,-29,-125b, -1229 - 141	(Z. Wang et al 2015), (Nie, J. et al., 2019), (Kwon, J. J. et al., 2019), (Liu, B. et al., 2018), (Tan, Z. et al., 2016), (Si, W. et al., 2016)
CD44	mixed	miR-205,-34a	(Ouhtit et al., 2007) (Tse, 2005) (Zhang, Lu. et al., 2020), (Ahir, M. et al., 2020)
CDCP1	oncoprotein	miR-198	(Wright, H. J. et al 2017) (Hu, Y. et al., 2017)
CEACAM-1/CD66a	tumor suppressor	miR-342	(Weng, C. et al., 2016) (C. Yang et al., 2017)
Claudin-1	oncoprotein	miR-155	(Zhou, B. et al., 2015) (Chiang et al., 2019)
COL1A1	oncoprotein	miR-196b-5p	(Zhu, X. et al.,2008), (Jiang, Y. et al., 2022) (W. Wu & Zheng, 2022)
CXCR4	oncoprotein	miR-9,-139	(Liu, Y. et al., 2021), (Cheng, C.-W. et al., 2021) (J. Li et al., 2021)
DDR1	oncoprotein	miR-199b-p	(Wu, A. et al., 2018) (Baltes et al., 2020)
JAM-A	mixed	miR-495, -145	(Cao, M. et al., 2014), (Ye, D. et al., 2019), (Naik et al., 2018), (Murakami et al., 2011)
JAM-B	tumor suppressor	miR-374	(Li, W. et al., 2019) (Bhan et al., 2013)
L1CAM	oncoprotein	miR-21-3p	(Doberstein, K. et al., 2014)
MUC1	oncoprotein	miR-200c, -141, -1226,	(Rajabi, H. et al., 2013), (Gao, Y. et al., 2016), (Kufe et al., 2010),
Nectin-4	mixed	miR-520c-3p	(Liu, Y. et al., (2022)) (Zeindler et al., 2019), (Sethy et al., 2018)

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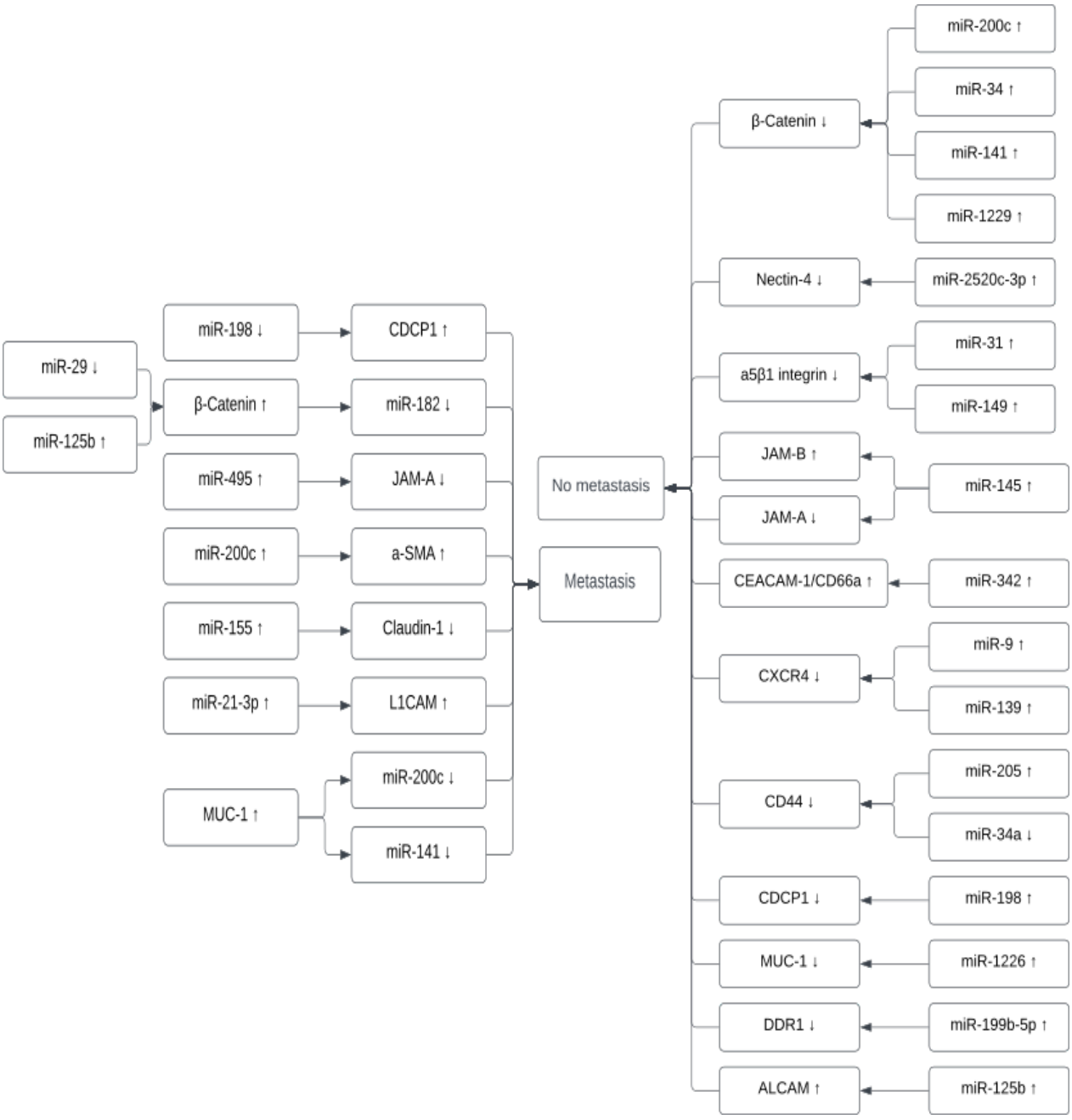

Figure 2. Description of relationship between miRNAs and epithelial markers in MBC

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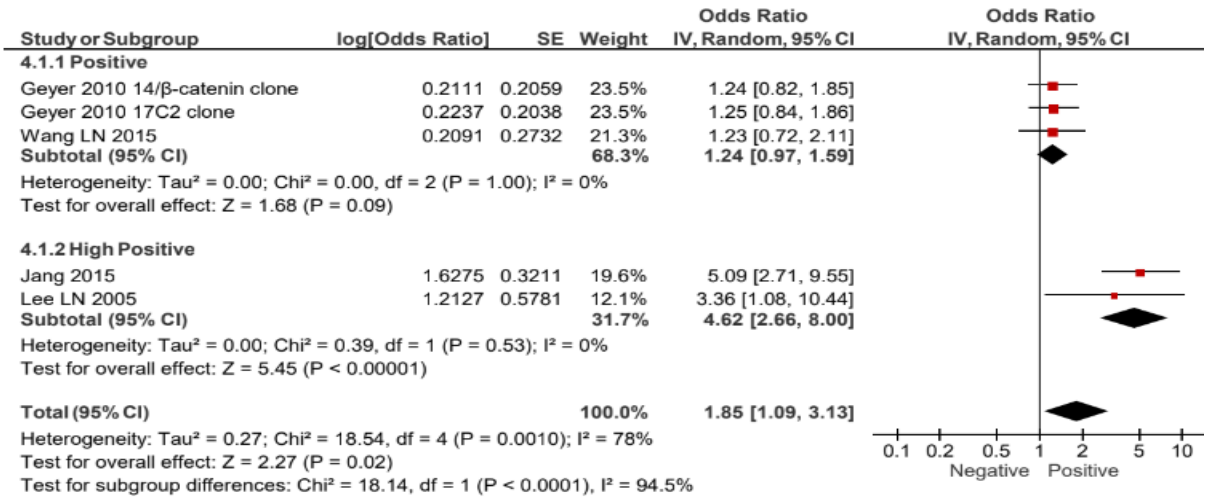

Figure 3. Meta-analysis on four studies assessing the association of β-catenin expression with the occurrence of MBC. The pooled OR was 1.85 (95% CI: 1.09-3.13; Z=2.27; P=0.02) with heterogeneity (I2 78% P=0.0010). Two studies showed mild positive correlation between B-catenin and the occurrence of MBC: Geyer (Geyer et al., 2011) and Wang (Z. Wang et al., 2015). The subtotal OR for the two studies was 1.24 (95 % Cl: 0.97-1.59; Z= 1.68(P= 0.09) without heterogeneity(I2 0% P=1). It should be noted that data from Geyer was used twice as he utilized two different types of B-catenin antibodies to prohibit metastasis. Two studies showed a high positive correlation between B-Catenin expression and occurrence of MBC: Jang (Jang et al., 2015) and Lee (Won-Lee, 2005). The subtotal OR was 4.62 (Cl% 95 2.66-8.00, Z= 2.27, P= 0.02) without heterogeneity (I2 0% P=0.53).

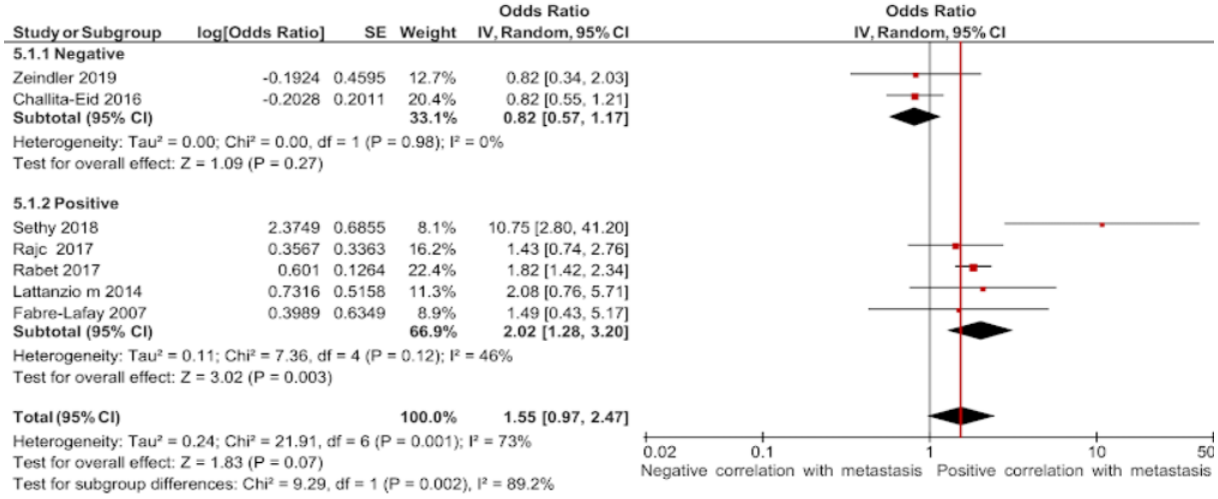

Figure 4. Meta-analysis on six studies assessing the association of Nectin-4 expression with the occurrence of MBC. The pooled OR was 1.55 (95% Cl: 0.97-2.47; Z= 1.83; P=0.07) with heterogeneity (I2 73% P= 0.002). Five studies showed positive correlation between Nectin-4 expression and the occurrence of MBC : Fabre-Lafay (Fabre-Lafay et al., 2007), Rabbit (M-Rabet et al., 2017), Lattanzio (Lattanzio et al., 2014), Sethy (Sethy et al., 2018), and Rajc (Rajc et al., 2017). The subtotal OR for the studies was 2.02 (95% Cl 1.28- 3.20 Z= 2.10; P= 0.04) with heterogeneity (I2=46%, P= 0.12). Two studies showed a negative correlation between Nectin-4 expression and the occurrence of MBC: Zeindler (Zeindler et al., 2019) and Chalita (Challita-Eid et al., 2016). The subtotal OR for those studies was 0.82 (95% Cl 0.82 Z= 1.09; P= 0.27) without heterogeneity.

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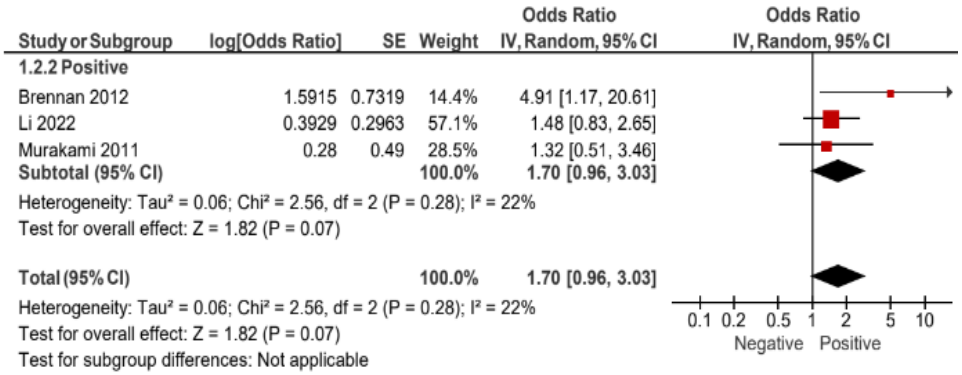

Figure 5. Meta-analysis on three studies assessing the association of JAM-A expression with the occurrence of MBC: Murakami (Murakami et al., 2011), ,Li (C.-H. Li et al., 2022), and Brennan (Brennan et al., 2013). The pooled OR was 1.70 (95% CI: 0.96-3.03; Z=1.82; P=0.28) with heterogeneity of 22% P=0.28. All three studies showed positive correlation between JAM-A and the occurrence of MBC.

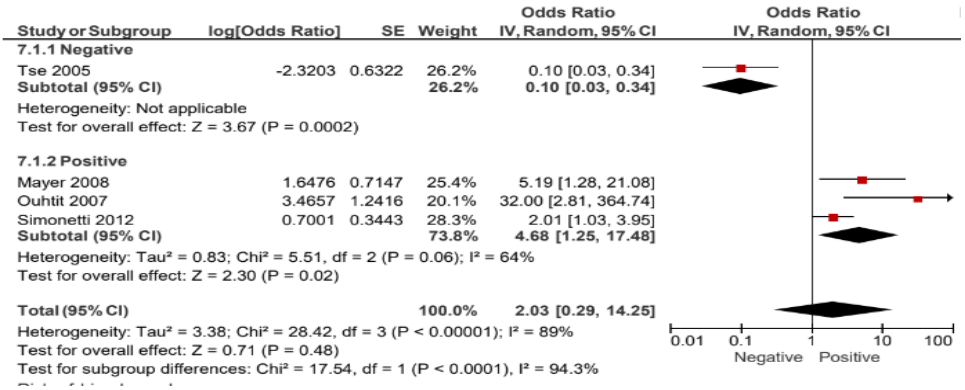

Figure 6. Meta-analysis on three studies assessing the association of CD44 expression with MBC. The pooled OR was 2.03 (95% Cl: 0.29 - 14.25; Z = 0.71; P=0.48) with heterogeneity (I2 89% P<0.00001). Three studies showed positive correlation between CD44 expression and the occurrence of MBC: Mayer (Mayer et al., 2008), Simonetti (Simonetti et al., 2012), and Ouhtit (Ouhtit et al., 2007). The subtotal OR for those studies was 4.68 (95% Cl 1.25 - 17.48; Z=2.30; P = 0.02) with heterogeneity 64%, P = 0.06. One study showed a negative correlation between CD44 and the occurrence of MBC: Tse (Tse, 2005). The subtotal OR for the study was 0.10 (95% Cl 0.10 Z= 3.67, P= 0.0002).

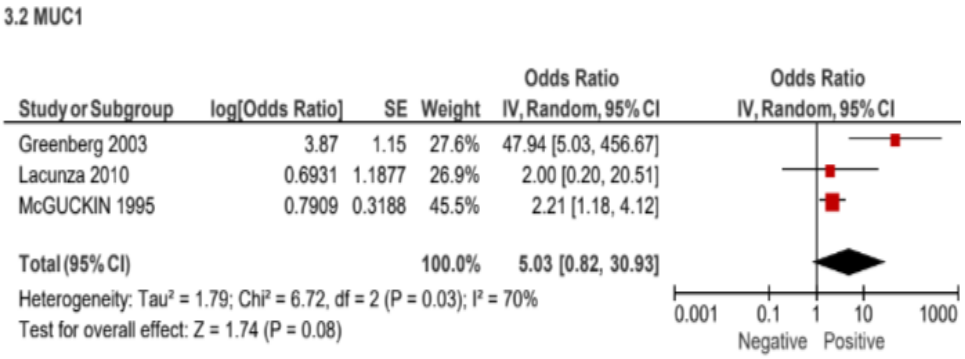

Figure 7. Meta-analysis on three studies assessing the association of MUC1 expression with MBC. The pooled OR was 5.03(95% Cl: 0.82 - 30.93; Z= 1.74; P= 0.08 with heterogeneity 70% P= 0.03. Three studies showed a positive correlation between MUC1 expression and the occurrence of MBC: McGuckin (Mcguckin, 1995), Greenberg (Greenberg et al., 2003), and Lacunza (Lacunza et al., 2010).

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3 Discussion

Studies are unanimous in the notion that β-catenin expression is positively correlated with MBC and that β-catenin is expressed in the nucleus and/or cytoplasm of breast cancer cells. Lack of consensus occurs regarding how apparent the correlation between β-catenin and MBC is. As the diamond representing the subtotal OR ratio for mild positive correlation crosses the horizontal line representing the 95% interval, it's likely that Geyer and Wang do not present a statistically significant result (Fig 3). This would be in accordance with the results reported by Wang, where he stated that although high expression of B-catenin is correlated with poor patient outcome, no statistically significant correlations was noticed between B-catenin expression and metastasis (Z. Wang et al., 2015). Nevertheless, Geyer, who received similar results to Wang claimed that aberrant nuclear B-catenin expression was significantly associated with lymph node metastasis, which we failed to show in our statistical analysis, as the odds ratio for Geyer crosses the 95% interval line (Fig 3) (Geyer et al., 2011). Possible reasons include that the study only included enough data to develop an odds ratio in the context of lymph node metastasis, and not lymph vascular invasion. The lack of heterogeneity between the two studies can be attributed to both Geyer and Wang reporting their results in the context of lymph node metastasis. Factors that could have limited the results of Geyer's study include that Wang collected data in a short period of time (2006-2007), which may not be long enough to observe metastasis in patients, and that the patients were treated with anthracycline-based chemotherapy. Future longitudinal investigations could provide invaluable insights into the temporal dynamics of β-catenin expression and its implications for metastasis. The administration of anthracycline-based chemotherapy among patients underscores the need to rigorously examine the potential influence of such treatments on β-catenin expression and its subsequent association with MBC. For Lee and Jang, which indicate high positive correlation between B-catenin expression and MBC, the diamond representing the subtotal OR didn't cross the 95% interval, leading to the conclusion that the results are statistically significant (Fig 3). A potential limitation of both studies would have been the small sample sizes used. However, since the results are statistically significant, one could conclude that the small sample size doesn't significantly undermine the results. Even though the studies exhibit no heterogeneity, the subtypes of breast cancer used in the two studies are different (Jang - Sca-1 positive and Lee - ductal breast carcinoma) (Jang et al., 2015), (Won-Lee, 2005). Lack of heterogeneity despite this factor may suggest a lack of significant correlation between the role of B-catenin in MBC, and breast cancer subtype.Studying the influence of β-catenin expression on different breast cancer subtypes could clarify its role in metastatic potentials. Studies not included in the meta-analysis due to lack of numerical data necessary to do an odds ratio and the cellular mechanisms through which B-catenin affects cancer described in the introduction, also support the notion that B-Catenin overexpression in the nucleus and/or cytoplasm correlates with MBC (Quinn et al., 2021), (Lin et al., 2000), (De et al., 2016).

The role of Nectin-4 in MBC has been a controversial topic in research with some studies suggesting that overexpression of Nectin-4 is negatively correlated with MBC, and others proposing that it is positively correlated with MBC (Fig 4). The subtotal and individual odds ratios of the studies which suggest negative correlation between Nectin-4 expression and MBC, all cross the 95% confidence interval line, suggesting that the results are not statistically significant (Fig 4). Despite the lack of heterogeneity between the results of the two studies (Fig 4), they discuss MBC in the context of different breast cancer subtypes (Zeindler - TNBC and Chelita - ductal and lobular) and used different antibodies to locate Nectin-4 (Zeindler- AGS-22M6, ASG-22C and Chelita - M22-244b3). Since prior studies on Nectin-4 have demonstrated its sensitivity to different types of antibodies, the homogeneity between the two studies was unexpected (Lattanzio et al., 2014). A comprehensive study focusing on how various antibodies impact the detection and quantification of Nectin-4 expression would be valuable.The subtotal OR ratio of the studies representing positive correlation between Nectin-4 and MBC didn't cross the 95% confidence interval line, indicating that the results from all the studies portrayed a statistically significant positive correlation between Nectin-4 expression and MBC (Fig 4). As indicated by the crossing of the subtotal OR ratio of Rajc with the 95% confidence interval line, the study failed to report a statistically significant result (Fig. 5). Such a conclusion would be consistent with the results explicitly stated by Rajc that MBC in HER2 negative breast cancer and Nectin-4 expression are not significantly correlated with one another (Rajc et al., 2017). Even though Fabre-Lafay and Lattanzio both reported significant correlation between Nectin-4 expression and MBC in TNBC and luminal A breast cancer, respectively,

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they both cross the 95% confidence interval line, indicating a statistically insignificant relationship (Fig 4)(Lattanzio et al., 2014), (Fabre-Lafay et al., 2007). Possible reasons for the difference between the results reported by them and those demonstrated by the statistical analysis include the limited amount of numerical data reported in Fabre-Lafey, and hence used in the statistical analysis, and the varied treatment the patients were subjected to in Lattanzio that could have additionally influenced metastasis. Rabet is a strong study, illustrated through its lack of cross with the 95% confidence interval line and high weight, due to its large amount of data (Fig 4). Sethy is also a strong study, with the only limitation being its heterogeneity from the other studies, likely resulting from its smaller dataset and assessment of ductal carcinomas, without specification of the molecular subtypes (Sethy et al., 2018). Except for Frabe Lafay who didn't specify the type of breast cancer carcinoma, all the other studies investigated Nectin-4 correlation in the context of a molecular subtype (Rabet - TNBC, Lattanzio and Rajc - luminal A). The inconsistency in data presentation suggests a need for standardized data collection and reporting methods to ensure comparability across studies.The study not included in the meta-analysis and the cellular processes outlined in the introduction also agree that Nectin-4 expression positively correlates with MBC (Shao et al., 2022).

The studies included in the meta-analysis all show JAM-A expression as having positive correlation with MBC. The pooled OR ratio crosses the 95% confidence interval leading to the conclusion that no statistically significant correlation between JAM-A expression and MBC can be observed (Fig 5). Murakami has a very low OR ratio meaning that the results reported aren't statistically significant, which is supported by inference made in the study (Murakami et al., 2011). Although Li reports similar results to Murakami, he states that JAM-A plays a role in several processes related to cell motility and is predominantly expressed in TNBC cells which are often associated with increased metastatic potential (Li et al., 2022). Since Li crosses the 95% confidence interval, the statistical analysis fails to reflect the reported results (Fig 5). Possible reasons include that in the study HER2 signaling and positive ER was perceived as a sign of metastasis, due to their causative relationship with TNBC. Nevertheless, to maintain homogeneity, the statistical analysis only considered the lymph node metastasis variable. A weakness of Li is the heterogeneity seen in the data pool with some patients being diagnosed in 1991. Brenan showcases a statistically significant positive correlation between JAM-A and MBC in Figure 5, supported by their own inferences in the study (Brennan et al., 2013). The heterogeneity of the data is in the acceptable range, as all the studies measure metastasis through lymph node involvement (Fig 5). Although different subtypes are used, limiting homogeneity, there are common subtypes used. For example, both Li and Brennan assess JAM-A expression in luminal A, luminal B, HER2 positive, and basal subtypes. Like Murakami, Li also assessed JAM-A expression in TNBC. The results are generally consistent with one another apart from Li who reported no correlation between JAM-A expression and MBC in basal breast cancer, whereas Brennan reported correlation between them. Brenan reported low expression of JAM-A in Luminal A breast cancer metastasis. However, Li united luminal A and B, and deduced positive correlation between JAM-A and metastasis in the subtype. More studies are needed to elucidate the role of JAM-A and metastasis in the context of luminal breast cancer subtypes. Overall, even though the three studies do not produce a statistically significant result, there are many more studies showcasing how JAM-A expression can lead to metastasis (Yang Wang and Lui, 2012), (McSherry et al., 2011) which weren't included due to lack of sufficient data to form OR ratios. There is also a study that shows how JAM-A expression can be negatively correlated with MBC (Naik et al., 2008) which we couldn't include due to the same reason. As there are explanations based on cellular mechanisms supporting both roles of JAM-A, inclusion of both types of studies would have yielded results with greater implications.

The role of CD44 has been disputed, with studies suggesting both its negative and positive correlation with MBC. The subtotal OR for the studies indicating positive correlation between MBC and CD44 doesn't cross the 95% confidence interval, meaning that the results are significant (Fig 6). However, the heterogeneity of the studies exceeds the expected range. Possible factors contributing to the heterogeneity of the results are the different ways through which MBC was measured through (Mayer - lymph node metastasis), (Ouhtit - metastasis to the liver), (Simonetti-number of invasive ductal/ micropapillary carcinomas) (Mayer et al., 2008) (Ouhtit et al., 2007) (Simonetti et al., 2012). The status of Ouhtit as an outlier can be attributed to them examining heterogeneity in vivo in mice, whereas the other two studies examined metastasis in patients. The heterogeneity among them suggests the sensitivity of CD44 to different tumor microenvironments and its context dependent nature, discussed in other studies, as well (Louderbough & Schroeder, 2011). To reduce heterogeneity, future studies could adopt a standardized method of

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measuring MBC. The OR ratio of the study that reported negative correlation between CD44 and MBC, didn't cross the 95% confidence interval indicating a statistically significant result (Fig 6). A weakness of the study is the difference between the age and tumor size in the control group with no observed metastasis, and the ones with, indicating that the two variables could have affected metastasis in addition to CD44 expression. Future research should ensure that control groups are matched carefully based on factors like age, tumor size, and other relevant parameters. This would provide a more accurate assessment of CD44's role without potential confounding variables.Tse only tested for MBC in the context of standard CD44, whereas the other three studies also tested for MBC in the context of CD44 variants such as CD44v5 and CD44v6, thereby suggesting a potential role of variants in the dual nature of CD44 in MBC.Future research should ensure that control groups are matched carefully based on factors like age, tumor size, and other relevant parameters. This would provide a more accurate assessment of CD44's role without potential confounding variables. A deeper investigation into the different CD44 variants (e.g., CD44v5 and CD44v6) and their individual or combined roles in MBC could distinguish whether certain variants have more pronounced effects on MBC than the standard CD44.More studies portraying negative correlation between CD44 would have strengthened the results, however (Lopez et al., 2005) didn't include sufficient numerical data.

Studies are unanimous in the notion that MUC1 expression positively correlates with MBC. However, as indicated by the subtotal OR ratio crossing the 95% confidence interval, the studies do not provide enough data for a statistically significant correlation (Fig 7). Although there were a total of 10 studies discussing the role of MUC1 in MBC, all leading to the conclusion stated above, we were able to find sufficient data for an OR ratio only in 3. Not only are 3 studies insufficient, but their individual sample sizes were also very small, further contributing to the statistical insignificance of the results. The data has heterogeneity in the acceptable range. However, it was unexpected that McGuckin and Lacunza have greater similarity between their results than Greenberg and Lacunza, since both Lacunza and Greenberg measured MUC1 expression in vivo, whereas McGuckin measured it in vitro (Greenberg et al., 2003) (Lacunza et al., 2010).

The data encompassed in this study facilitated the contextual interpretation of both the meta-analysis outcomes and the identification of epithelial markers implicated in MBC, highlighting the need for marker comparisons. The meta-analysis findings not only suggest new research avenues to refine our understanding of the epithelial markers' role in MBC, but also offer a critical evaluation of current literature.

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