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Literature Review

ROLE OF HYPOXIA INDUCIBLE FACTOR-1α IN LYMPH NODE METASTASIS OF BREAST CANCER

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ABSTRACT

Background: Lymph node metastasis in breast cancer is a clinical parameter used to predict a patient's prognosis. Lymph node metastasis is affected by numerous factors, one of which is hypoxic conditions that trigger the expression of HIF-1α, a transcription factor expressed by cells in hypoxia. To date, the role of HIF-1α in lymph node metastasis in breast cancer requires further research.

Objective: This review aimed to describe the possible role of HIF-1α in breast cancer lymph node metastasis.

Methods: PubMed and Google Scholar databases were searched for articles using Boolean operations with keywords related to HIF-1α expression and lymph node metastasis of breast cancer. We have limited the 10 years of publication to be included in this review.

Results: HIF-1α has the potential to induce epithelial-mesenchymal transition, extracellular matrix invasion, matrix degradation, and lymphangiogenesis through various target gene expression and signaling pathways, leading to lymph node metastasis of breast cancer cells.

Keywords: hypoxia, HIF-1α, lymph node metastasis, and breast cancer

INTRODUCTION

Breast cancer is one of the most common malignancies in the world.[31] Breast cancer is a type of epithelial cancer capable to metastasized into various organs, one of which is the lymph nodes.[1,2,31] Lymphatic vessels differ from blood vessels in their ability to provide an oxygen supply to support tumor growth. Lymph nodes may lack this capability, but they can provide an easier metastatic route for breast cancer cells.[19] Lymphatic vessels have a thin wall structure and high permeability, so they can easily penetrate breast cancer cells.[19] For this reason, early lymph node metastasis is one of the marker used by clinicians to determind the prognosis of breast cancer patients.[31,32,35]

Breast cancer initiation, progression, and metastasis to lymph nodes are influenced by three factors: somatic mutations involving oncogenes and tumor suppressor genes, epigenetic alterations that cause changes in gene expression patterns, and the tumor microenvironment.[2] The tumor microenvironment is divided into chemical components such as pH, PO2, metabolites, and cellular components such as tumor cells, extracellular matrix produced by tumor cells, lymphatic endothelial cells, and many more. Chemical and cellular components can interact with each other to support tumor growth and development.[2]

Breast cancer is a type of solid tumor with a high proliferation capacity, so it can be large in size.[3] The blood vessels in the human body can support the growth of cancer cells within a maximum volume of 2 mm.[3,4] Breast cancer cells that grow more than this volume then become hypoxic. Intratumoral hypoxia in breast cancer triggers the activation of the transcription factor hypoxia inducible factor 1 (HIF-1) as an adaptive mechanism.[5]

Hypoxia Inducible Factor-1 (HIF-1) is a specific heterodimer transcription factor composed of two subunits, the alpha (α) subunit that has the capability to sense changes in oxygen levels and the beta (β) subunit that plays a role in translocating the heterodimer complex into the nucleus.[6,30] Under normoxic conditions, HIF-1α has a short lifetime, only around 5 minutes, because HIF-1α binds to oxygen, is hydroxylated, and is degraded by the proteasome system.[6,30] In conditions where oxygen is low or hypoxic, HIF-1α cannot bind to oxygen and is recognized and hydroxylated, which leads to inactivation of the proteasome degradation system.[30] Undegraded HIF-1α forms a bond with HIF-1β, forming a heterodimer complex structure that translocates into the nucleus.[30] The HIF-1α/β heterodimer complex binds to protein p300 to form an active transcription complex, which then binds to a specific DNA sequence called the hypoxia response element (HRE) in target genes. The binding of the HIF-1α/β complex to the HRE triggers the transcription of target

genes, such as those involved in lymph node metastasis of breast cancer.[6,30] Various studies have attempted to show the important roles of HIF-1α in lymph node metastasis in breast cancer, but these studies still deliver inconsistent results (Table 1).

Table 1. Comparison of many studies.

Author	Years of publication	Sample size	Observed parameters	Methods	Studies result
Cai et.al.	2016	297	Age, tumor size, skin involvement, lymph node metastases, histological grade, tumor size, clinical stage, hormone receptor, survival, HIF-1α	ELISA using plasma samples	HIF-1α expression is significantly correlated with lymph node metastases, tumor size, skin ivolvement tumor type, and clinical stage but not an independent prognostic factor for breast cancer patient's survival.
Kozakiewicz et.al.	2016	58	Age, histological type of invasive breast cancer, HIF-1α expression, EPO-R expression, and steroid receptor expression	Immunohistochemistry to measure HIF-1α expression, EPO-R expression, and steroid receptor expression	There was no significant correlation between HIF-1α expression and the incidence of lymph node metastasis in breast cancer although HIF-1α expression was still found in 36.2% of breast cancer samples.
Sharma et. al.	2017	50	Age, tumor size, histological grade, lymph node status, HIF-1α expression, BRCA1 positivity, VEGF	Immunohistochemistry to measure HIF-1α expression and BRCA1 positivity, ELISA for VEGF serum sample level measurement	HIF-1α expression, BRCA1 positivity, and increased VEGF levels were significantly associated with higher grade and lymph node metastasis
Nie et.al.	2018	220	Age, tumor size, nodal status, HR, Her-2, Ki-67, molecular subtype, chemotherapy type, surgery type, HIF-1α	Immunohistochemistry in patients stages II to III invasive breast cancer patient with neoadjuvant therapy	Statistically significant correlation between increased HIF-1α expression and positive lymph node status in locally advanced breast cancer.
Cui and Jiang	2019	87	Age, tumor size, histological grade, lymph node, TNM stage, Ki-67, HIF-1α, c-myc	Immunohistochemistry in Triple Negative Breast Cancer patient tissue	Increased HIF-1α expression correlated significantly with positive lymph node status (p = 0.007)
Zhao et.al.	2020	5177	Clinicopatholocial characteristics, survival of breast cancer patient, HIF-1α expression	Meta Analysis using Cochrane Library, Web of Science, PubMed, EMBASE online database	Strong correlation between HIF-1α expression and positive lymph node status (p = 0.002) and a pooled odds ratio of 1.646.
Gunawan et. al.	2020	40	Age, breast cancer type, histological grade, hypoxia associated biomarkers (CAIX and HIF1A)	Immunohistochemistry and RT-qPCR analysis for measuring hypoxia associated biomarker mRNA	HIF-1α expression does not correlate with patient age, tumor advancement (locally advanced and metastatic), hormonal status, and does not have a significant prognostic value for the overall survival of breast cancer patients.
Hegde et.al.	2022	65	Altered genes expressions in metastases breast cancer	Micro array and immunohistochemistry	STAT3/HIF1A genes expression are significantly increased and correlated with lymph node metastases risk through VEGFR3/VEGFD pathway.

Table 1. *Continue*

Author	Years of publication	Sample size	Observed parameters	Methods	Studies result
Chen et.al.	2024	124	Age, tumor size, vascular tumor thrombus, histological grade, Ki-67, lymph node metastasis, HIF-1α expression, CD147 expression, prognosis	Immunohistochemistry to look the location of HIF-1α and CD147 expression in the cell, qPCR to measure the expression of HIF-1α and CD147	The expression of HIF-1α and CD147 was significantly higher in TNBC. HIF-1α expression was significantly correlate with histological grade and lymph node metastasis in TNBC but not significant with patient's prognosis.
Lian et. al.	2020	175	Age, tumor size, lymph node metastasis, lymphatic invasion, histological grade,	Immunohistochemistry was used to detect the expresion of HIF-1α, BRD4.Beclin1, LC3B, and p62 expression. These protein expression were also measured after 24 hour stimulation in breast cancer cell lines.	High expressions of HIF-1a and Beclin1 were significantly correlated with lymph node metastasis and lymphatic invasion. The expression of HIF-1α, BRD4, Beclin1 and LC3B was up-regulated in breast cancer cells after 24 hours hypoxic stimulation.

These inconsistent results may be caused by the different methods used in many studies and different cutoff points for interpreting HIF-1α expression. To the best of our knowledge, there is no consensus on the best method supposed to be used to analyze HIF-1α expression and the exact cut-off point to classify HIF-1α expression as high, normal, or low until today.[24]

Even though there is diversity in research results, various studies have been conducted before agreed that HIF-1a is a transcription factor that may play an important role in the process of lymph node metastasis of breast cancer and has the potential to serve as a biomarker of lymph node metastasis in breast cancer.

METHOD

This review was conducted through a literature search of the PubMed and Google Scholar databases of journals. The keywords used were "hypoxia", "hypoxia inducible factor-1alpha", "HIF-1alpha", "HIF-1α", "HIF1A", "lymph node metastasis",and "breast cancer". The keywords were combined using Boolean operations, and the literature was found to have a maximum of 10 years of publication. Inclusion criteria include original research articles related to the role of hypoxia-inducible factor-1α in lymph node metastasis of breast cancer and its molecular mechanism, as well as the signaling pathways involved in this mechanism. We minimized the number of review articles to reduce bias. Exclusion criteria included literature in languages other than English, abstract, or full text not accessible to researchers, gray literature (unpublished theses, dissertations, reports, guides, seminar papers, and symposiums), and literature discussing the role of hypoxia-inducible factor-1α in non-primary breast cancer tumors. There total of 3332 article appeared using combination keywords. After applying the inclusion and exclusion criteria, we found 10 articles that matched our criteria. Analysis and synthesis were then carried out in a narrative manner by summarizing the collected literature and subsequently grouping it according to the possible role of hypoxia-inducible factor-1α in lymph node metastasis in breast cancer.

RESULTS AND DISCUSSIONS

Hypoxia Inducible Factor-1 α and EMT

To begin the metastasis process, epithelial cancer cells must escape from the primary tumor nest and attach to and invade the surrounding mesenchymal tissue before entering the lymph nodes.[16] During the process of escaping from the primary tumor nest, epithelial cancer cells need to transform themselves into mesenchymal cells which are part of the extracellular matrix through a process called epithelial-mesenchymal transition (EMT).[16,17,29] EMT begins with the loss of epithelial molecular markers, such as E-cadherin, and an increase in mesenchymal molecular markers such as vimentin.[16,17] This phenomenon induces epithelial cancer cells to lose their polarity and cohesion, causing cells that originally had an epithelial phenotype to transform into a mesenchymal phenotype.[16,17] Research by Shao et.al. (2015) showed that increasing HIF-1α expression together with increasing Brachyury expression suppressed E-chaderin expression and increased vimentin expression in breast cancer. Brachyury expression correlates with tumor stage, tumor size, and lymph node metastasis.[17] Brachyury expression is modulated by hypoxic conditions in breast cancer; therefore, it is possible that HIF-1α expression indirectly influences lymph node metastasis of breast cancer through the induction of EMT in the Brachyury transcription pathway.

Hypoxia Inducible Factor-1 α and Extracellular Matrix Invasion

Extracellular matrix invasion begins when transformed cancer cells interact with the extracellular matrix to

reduce bonds and adhesions between cancer cells to facilitate the movement of cancer cells into another areas.[17,34] Tumor cells attach to the extracellular matrix through various proteins including integrin.[5] Integrins mediate the bond between breast cancer cells and collagen or fibronectin. The expression of integrins that bind to collagen is regulated by ITGA1, ITGA 11, and ITGB1, while the expression of integrins that bind to fibronectin is regulated by ITGA5 and ITGB1.[5] Ju et.al. (2017) found that HIF-1a expressed under hypoxic conditions binds to the hypoxia response element (HRE) in the ITGA5 promoter, thereby triggering the protrusion of actin protein spikes in the 3D gel medium and facilitating single cancer cells to migrate to the lungs and lymph nodes. Integrin expression is also known to increase with the combined activity of HIF-1a and PFKFB4, a gene that codes for metabolic adaptation to hypoxic conditions, as well as cell plasticity and the appearance of an invasive phenotype in cancer.[18] Dai et.al. (2022) showed that increasing HIF-1a expression induces PFKFB4 translocation to the nucleus, causing increased integrin expression at both the mRNA and protein levels.

Hypoxia Inducible Factor-1 α and MMPs

Breast cancer cells have to degrade the basement membrane and various matrix barriers to invade lymph nodes.[20] Matrix metalloproteinases (MMPs) type 2 and 9 are endopeptidases that can degrade type 4 collagen in the basement membrane.[20] Many studies have shown that hypoxic conditions and HIF-1α expression can increase the expression and activity of MMP-2 and MMP-9 in breast cancer.[16,20] Disintegrin and metalloprotease (ADAM), a large family of membran integral glycoptroteins that regulate cell adhesion and migration, is one of HIF-1α target genes.[28] Increased expression of ADAM12 in an HIF-1α dependent manner is known to increase breast cancer motility and lymph node metastasis.[28] Increased expression and activity of these MMPs are also known to be positively correlated with poor prognosis in breast cancer patients.[16,20]

Hypoxia Inducible Factor-1 α and Lymphatic Endhotelium

Lymph node metastasis in breast cancer is influenced not only by tumor cell activity under hypoxic conditions but also by the response of lymph node endothelial cells in pre-metastatic organs. Like an orchestra, tumor cells secrete various factors that trigger the secretion of CCL5, a pro-inflammatory chemokine, and vascular endothelial growth factors (VEGFs), which facilitate tumor cells to escape from the primary tumor nest, extravasate, and form colonies in the lymph nodes.[21] Tumor cells also express IL6, which activates the Stat3 pathway in the lymph node endothelium and results in increased expression of CCL5 and VEGFs.[21] Increased expression of HIF-1α under hypoxic conditions is known to increase the expression of VEGF-A, VEGF-C, and VEGF-D, which play a role in lymph node metastasis in breast cancer.[19,21] VEGF-A is a glycoprotein that plays a role in the migration of endothelial cells, vascular endothelial cells, and lymphatic endothelial cells, depending on which receptor they bind to.[19] Hypoxic conditions are known to increase the rate of translation of VEGF-A mRNA, leading to increased expression of VEGF-A. Increased VEGF-A triggers increased binding of VEGF-A with VEGFR2/R3 to stimulate the formation of new lymph vessels (lymphangiogenesis).[19]

However, the role of VEGF-C in lymph node metastasis in hypoxic breast cancer remains controversial. VEGF-C is an essential protein that plays a role in the lymphangiogenesis through the VEGF-C/VEGFR3 signaling pathway.[19,33,36] VEGF-C expression is increased under hypoxic conditions; however, VEGF-C is also known to not have an HRE, the site of attachment for HIF-1α.[19,33] Lee et.al.(2015) investigated this and found that VEGF expression could be induced by the activation of IL6-pStat3 signaling in hypoxic conditions. Although this signaling pathway also increases HIF-1α expression, more in-depth research is required.

VEGF-D is a protein that is able to bind to VEGFR-3 to promote lymphangiogenesis and lymph node metastasis in various cancers including breast cancer.[19] In invasive carcinoma breast cancer, the expression of VEGF-D is correlated with the expression of HIF-1α; however, how this process plays a role in lymph node metastasis of breast cancer is still unclear. Taken together, HIF-1α is a transcription factor that regulates the expression of various genes and signaling pathways, leading to lymph node metastasis in breast cancer (Figure 1).

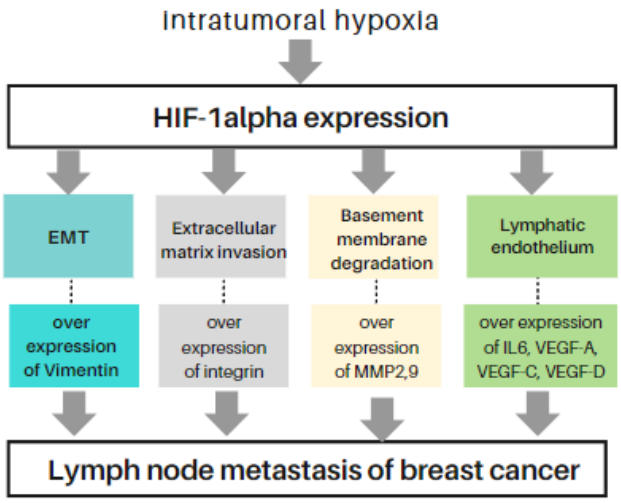

Figure 1. Mechanism of HIF-1α involved in lymph node metastasis of breast cancer

CONCLUSION

HIF-1α is a transcription factor with the ability to induce epithelial-mesenchymal transition, extracellular matrix invasion, matrix degradation, and lymphangiogenesis through the expression of its target genes, leading to lymph node metastasis in breast cancer cells. The use of HIF-1α as a potential biomarker of early lymph node metastasis in breast cancer to improve patient prognosis still needs further research, as there have been inconsistent results in many studies.

CONFLICT OF INTEREST AND FUNDING RESOURCES

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