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Review

Leukemia inhibitory factor: A main controller of breast cancer

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Breast cancer is the second leading cause of cancer-related death among females, worldwide. The cytokines are proteins that have a significant role in the development of tumor growth. Leukemia inhibitory factor (LIF) of the interleukin-6 cytokines superfamily plays a significant role, by the modulation of many signaling pathways. This study summarizes some current works in breast cancer, in which LIF intervention is being discussed. LIF promotes tumorigenesis, invasion, migration of breast cancer cells *in vitro*, metastasis of breast cancer *in vivo*, epithelial-mesenchymal transition, and mediates pro-invasive activation of stromal fibroblast. LIF contributes to inducing growth, tumorigenesis, and metastasis of breast cancer and is a significant biomarker for breast tumors and can be a therapeutic target for clinical intervention.

Keywords. Breast cancer; epithelial-mesenchymal transition; leukemia inhibitory factor; metastasis; proliferation

1. Introduction

Breast cancer as a global health problem is one of the most common malignant tumors and the second leading cause of death from cancer, after lung cancer in the United States. However, with progress in prevention, surgical resection and auxiliary therapies, the breast cancer death rate has been declining, although about 268,600 new cases and 41,760 deaths in the United States are reported in 2019 (DeSantis *et al.* 2019). Eighty-one percent of breast cancers are diagnosed, among women who have more than 50 years old, and eighty-nine percent of deaths happen in this age group. Metastasis to vital organs like lung, bone and brain is the main cause of mortality in breast cancer (Nguyen *et al.* 2009). Despite the fact that surgery, chemotherapy and radiation therapy can control many localized cancers, metastasis is still a dilemma (Kozłowski *et al.* 2015; Samandari *et al.* 2018; Wan *et al.* 2013).

There are many risk factors associated with breast cancer development, including sex, obesity, older age, genetics, lack of physical exercise, lack of maternity, alcohol consumption, higher levels of estrogens, radiation exposure, diabetes, positive family history, tobacco smoking and early age at menarche (Anothaisintawee *et al.* 2013; Gøtzsche and Jørgensen 2013; Johnson *et al.* 2011; I-Lee *et al.* 2012). A genetic mutation may play a main role in developing breast tumor, such as mutations in *BRCA1* and *BRCA2*, *P53* (Li–Fraumeni syndrome), *STK11* (Peutz–Jeghers syndrome) and *PTEN* (Cowden syndrome) (Gage *et al.* 2012; Nandikolla *et al.* 2017; Tsang *et al.* 2013). The RAS/MEK/ERK and phospho-inositide 3-kinase (PI3K/AKT) pathways maintain normal cells from self-destruction. Mutation in the genes

encoding these protective pathways results in cell survival, once no longer are needed, leading to the progression of cancer (Cavalieri *et al.* 2006).

Alterations in the growth factor signaling develop malignant cell growth. Leptinin overexpression increases cell proliferation, leading to breast cancer (Jardé *et al.* 2011). Alteration in the expression of a signaling molecule has a significant role in the activation of PI3K/AKT pathway, inactivation of mitogen-activated protein kinases (MAPK) pathway, and developing breast cancer (Guille *et al.* 2013). One of these signaling molecules is leukemia inhibitory factor (LIF) that mediates critical signaling pathway, including the Janus tyrosine kinase/signal transducer, activator of transcription 3 (JAK/STAT3), PI3K signaling pathways and p44/42 mitogen-activated protein kinase (ERK1/2) (Burdon *et al.* 2002). The objective of the present article is to review the intervention of the LIF in breast cancer as a possible therapeutic target.

2. Leukemia inhibitory factor (LIF)

LIF with a molecular weight of 38 to 67 kDa is the glycoprotein cytokine, belonging to the interleukin-6 cytokine superfamily, which includes IL-6, oncostatin M, IL-11, cardiotrophin-1, ciliary neurotrophic factor, and cardiotrophin-like cytokine (Boulton *et al.* 1994). LIF with 180-amino-acid, as a multi-functional protein, acts in various tissues and cells, through the activation of various signaling pathways (Gearing 1993).

Overexpression of human LIF has been shown in the circulatory system or body fluids (Mashayekhi and Salehi 2011) and various tissues, including the thymus, lung (Fukada *et al.* 1997), hypophysis (Chesnokova and Melmed 2000), cardiac muscle (Ancey *et al.* 2002), kidney (Morel *et al.* 2000), skin (Bonifati *et al.* 1998), uterine gland cells (Song *et al.* 2000; Vogiagis and Salamonsen 1999), neuronal tissue (Guang Ren *et al.* 1998; Ren *et al.* 1999; My Thum *et al.* 2006), and tumor tissues such as breast cancer (García-Tuñón *et al.* 2008; Kuphal *et al.* 2013), nasopharyngeal carcinogenesis (Liu and Chang 2014), oral squamous cell carcinoma (Ohata *et al.* 2018), ovarian cancer (K McLean *et al.* 2019), pancreatic cancer (Bressy *et al.* 2018), colorectal cancer (Liu *et al.* 2015) and malignant melanoma (Kuphal *et al.* 2013). Elevated levels of the LIF have been found in cases of inflammation (Gadient and Patterson 1999), blastocyst implantation (Paiva *et al.* 2009), autoimmune diseases and cell proliferation (Kellokumpu-

Lehtinen *et al.* 1996). LIF can be produced by immune cells, melanomas (Mattei *et al.* 1994), stimulated T-lymphocytes, stimulated monocytes, carcinoma cell lines (Kamohara *et al.* 1994), stromal cells and cancer-associated fibroblasts (Ohata *et al.* 2018). LIF production can be induced at mRNA level by different elements, such as inflammatory factors in different cells (Auernhammer and Melmed 2000; Knight *et al.* 1999; Palmqvist *et al.* 2008; Sherwin *et al.* 2004; Umemiya-Okada *et al.* 1992; Wetzler *et al.* 1991). The LIF expression is also regulated by estrogen and p53 in uterine tissues at the implantation stage (Sherwin *et al.* 2004). In this stage, the estrogen levels were elevated and caused the overexpression of the LIF mRNA in the uterine tissues that is vital for the implantation. In addition, the LIF expression at the implantation stage requires P53. There are a consensus P53-binding element on the *LIF,* controlling the LIF expression in uterine tissues (Yue *et al.* 2015).

Biological actions of the LIF are mediated by binding to the LIF receptor complex that is made up of glycoprotein gp130 and LIF receptor (LIF-R) subunit (Gearing *et al.* 1993). This binding activates distinct signaling pathways, including JAK/STAT3, MAPK, PI3K/AKT, ERK1/2 and mTOR pathways (Arthan *et al.* 2010; Heinrich *et al.* 1998; Slaets *et al.* 2008). The first known ability of LIF is murine M1 myeloid leukemia differentiation and macrophage maturation that prevent leukemia proliferation (Gearing *et al.* 1987a, b). LIF may be a hematopoietic regulator (Metcalf 2003) and potentially have a specific suppressive activity on some myeloid leukemia (Gearing *et al.* 1987a, b; Hilton *et al.* 1988). It has been confirmed that LIF animates the multiplication of factor-dependent hematopoietic and murine leukemic cell lines (Laâbi *et al.* 2000; Moreau *et al.* 1988). Further, LIF injected into mice led to an increase in the number of megakaryocyte and platelet cells after 7–10 days (Metcalf *et al.* 1990). LIF also increased calcium to albumin ratios and raised erythrocyte sedimentation rate in the serum (Mayer *et al.* 1993). LIF as a crucial regulator of human embryonic development plays a critical role in the implantation of the developing embryo, such as the receptive condition of endometrial, endometrial and embryo interaction, blastocyst invasion and the penetration of uterine leukocyte (Arici *et al.* 1995). It has been shown that LIF$^{-/-}$ females were not able to become pregnant (Escary *et al.* 1993; Stewart *et al.* 1992). Probably the reason for this defect is the vital lack of estrogen-induced LIF synthesis in the uterine wall when implanting blastocysts (Chen *et al.* 2000; Croy *et al.* 1991). Implantation

of LIF$^{-/-}$ blastocyst in a LIF$^{+/+}$ uterus forms a normal embryo and LIF$^{-/-}$ mice can become pregnant by LIF injection (Chen *et al.* 2000).

On the other hand, this is an important factor in the mouse embryonic stem cell growth (Dimitriadis *et al.* 2010) and maintain the pluripotentiality of murine embryonic stem cells (Smith *et al.* 1988; Thomson *et al.* 1998; Williams *et al.* 1988). The best-known ability of LIF is the inhibition of the differentiation of embryonic stem cells in mice through activation of the STAT3 pathway, causing self-renewal of stem cell, stimulation of proliferation of mouse primordial germ cells and induction of proliferation of myoblast (Cheng *et al.* 1994; Niwa 2001). Additionally, LIF affects the endocrine, reproductive, inflammatory and immune systems (Taga and Kishimoto 1997), and leads to the development of malignancies such as rhabdomyosarcoma, choriocarcinoma and melanoma (Fitzgerald *et al.* 2005; Maruta *et al.* 2009; Wysoczynski *et al.* 2007). The LIF overexpression enhances calcium resorption from bone and raises osteoclast numbers (Dazai *et al.* 2000; Reid *et al.* 1990). By binding LIF to the surface of osteoblast cells, it stimulates bone formation and results in bone density. It has been shown that LIF increases the maintenance of sensory and motor neurons (Murphy *et al.* 1991) and affects the formation and the proliferation rate of sensory neurons from neural crest cells (Carpenter *et al.* 1999). In addition, LIF averts oligodendrocyte death in multiple sclerosis animal models (Butzkueven *et al.* 2002) and elevates the migration of inflammatory macrophages in damaged neuronal tissue (Sugiura *et al.* 2000). In early cellular response to neural damage, LIF is an essential proinflammatory factor and this may be attributed to a direct chemotactic effect on inflammatory cells.

3. LIF and LIFR levels in breast cancer

The LIF overexpression has been shown at the mRNA and protein levels in human breast cancer (Dhingra *et al.* 1998; Kellokumpu-Lehtinen *et al.* 1996). The LIF level can be regulated by progestins and antiprogestin (Bamberger *et al.* 1998), and influences the proliferation of fresh breast carcinoma cells and some estrogen-dependent (MCF-7 and T47D), and estrogen-independent (SK-BR3 and BT20) breast cancer cell lines (Estrov *et al.* 1995). The hypoxia condition in solid tumors induces the stabilization of hypoxia-inducible factors (HIFs), and causes hypoxia responses in the cells (Keith and Simon 2007). HIFs

bind to DNA, harboring a hypoxia-responsive element (HRE; 5′-G/ACGTG-3′) and can control the transcription of the associated target genes (Wu *et al.* 2015). The level of LIFR, gpl30 and LIF mRNA has been shown controversial results in different laboratories. This may be due to the genetic and biological differences between the various subtypes of the MCF-7 cell. In a study, it was found that MCF-7 cells expressed the LIFR and gpl30 only. The PCR and ELISA results showed that LIF is not expressed in the cell lysates or in the media, suggesting that LIF does not increase growth through the autocrine pathway for MCF-7 cells (Estrov *et al.* 1995). The other group of researchers observed a high expression of LIFR mRNA, LIF mRNA and LIF protein in primary breast tumors. Interestingly, these researchers observed that the LIF inhibits the MCF7 cell growth, compared to MDA- MB-231, BT-549 and T-47D cells (Douglas *et al.* 1997).

Dhingra *et al.* evaluated the LIF and LIFR expression in 50 human breast cancer specimens. For *in situ* detection of LIF/LIFR, they developed immunohistochemical techniques and the expression was observed about 78% and 80% in tumors, respectively. These results indicate that LIF and LIFR are greatly expressed in breast tumors, compared to the normal specimen and their expression correlates with desirable biological characteristics of breast tumors (Dhingra *et al.* 1998). The LIF mRNA levels have been evaluated in several human breast cancer cell lines, such as T47D, MCF7, SK-Br-3, HS578T, MDA-MB-232, BT474, and MDA-MB-468 cells (Li *et al.* 2014). The LIF expression level fluctuates among these cell lines, correlating with cell line metastatic ability. In HS578T and MDA-MB-231 cells that exhibit higher metastatic abilities (Zajchowski *et al.* 2001), the LIF expression level is much higher than other cell lines with less metastatic ability. Chen *et al.* assessed the expression level of LIFR protein in human breast cancer cell lines. The expression of LIFR was higher in non-metastatic tumor cell lines MCF7, SUM159, SUM149, SUM229 and T47D, but was lower in the metastatic cell lines (SUM1315 and MDA-MB-231). They also examined the miR-9 expression in these cell lines and found that miR-9 promoted metastasis by affecting the metastasis suppressor E-cadherin (Ma *et al.* 2010), and showed that downregulation of LIFR is correlated to the miR-9 expression, and metastatic ability and loss of LIFR promote the metastasis effects of miR-9 in E-cadherin-negative breast cancer cells (Chen *et al.* 2012).

4.   Functional role of LIF in breast cancer

4.1   *LIF enhances breast cancer tumor growth*

In an investigation, MCF7 cells interacted with radiolabeled LIF; binding of LIF to its receptor was specific and depended on period, amount and temperature (Estrov *et al.* 1995). Incubation of cells with LIF after 72 hours promoted the total number of viable adherent and non-adherent MCF7 cells three times, and the LIF effect was less on the normal breast epithelial cell. In addition, there are morphological differences between LIF-incubated and unincubated cells. In the absence of LIF, cells were mainly adherent; however, in the presence of LIF, there was an elevation in the viable non-adherent population (Estrov *et al.* 1995). The role of exogenous LIF on the primary tumor cell growth in methylcellulose cultures was studied, showing colony growth was increasing 12–52% at a concentration of 40 ng/mL LIF (Dhingra *et al.* 1998). It has been proposed that this growth could be the result of DNA synthesis stimulation by LIF. In the other investigation, immunohistochemistry results showed, *in situ* carcinoma expressed the highest level of LIF, OSM and OSMRβ, compared to infiltrating tumors and benign breast lesions. They found that the expression of LIF, OSM, gp130, LIFRβ and OSMRβ increased by the progression of breast tumor, and this correlated to the malignancy. The LIF and OSM expression can develop tumor epithelial cell growth and act as a growth factor in breast cancer through a paracrine or autocrine pathway (García-Tuñón *et al.* 2008). Previous research has shown that both OSM and LIF effect on breast cancer biology due to that LIFRβ and OSMRβ are able to activate STAT3, and activation of STAT3 is associated with the malignant phenotype (Turkson and Jove 2000). These effects lead to cell proliferation and suppressing of the apoptosis through Bcl-X_l upregulation (Catlett-Falcone *et al.* 1999).

Li and his colleagues assessed the ectopic and the endogenous LIF expression in breast cancer, they found that ectopic LIF expression enhanced the MDA-MB-231, T47D, and MCF-7 cell proliferation, through activation of the AKT-mTOR signaling pathway (Fig.1). The LIF knockdown, using an AKT inhibitor, wortmannin, decreases the MDA-MB-231 cell growth, and inhibits the activation of AKT that leads to disruption of mTOR activation, and blocks the tumorigenesis effect of LIF (Li *et al.* 2014). Kellokumpu-Lehtinen investigated the role of LIF on proliferation of MCF-7 and T-47D breast cancer cell lines. LIF increased MCF-7 cell colony growth significantly at 40 ng/mL and 80 ng/mL concentration, and T-47D proliferation at 20 ng/mL. Anti-LIF-neutralizing antibodies decreased MCF-7 and T47-D cell proliferation. This change was observed in media without serum and estrogen. This shows that LIF is not dependent on the presence of estrogen or any growth factor and cytokine (Kellokumpu-Lehtinen *et al.* 1996). On the other hand, LIF did not induce the proliferation of MDA MB-231 breast cancer cells (Kellokumpu-Lehtinen *et al.* 1996).

4.2   *LIF inhibits normal breast epithelial cell proliferation*

The proliferation of non-malignant human breast epithelial cells (HBECs) was inhibited after LIF (10 ng/d) and OSM (10 ng/ml) therapy, separately. Interestingly, the presence of a culture medium, containing mitogen and epidermal growth factor (EGF) is necessary for *in vitro* culture of HBECs. In spite of the presence of breast cell mitogens, the inhibitory role of OSM and LIF was observed in normal breast epithelial cells (Grant *et al.* 2001). It has been observed that cells decreased in the S-phase of the cell cycle and accumulated in the G_0/G_1 cell cycle and thereby suppressed cell proliferation.

4.3   *LIF promotes breast cancer metastasis*

LIF enhances breast cancer cell invasion and metastasis, through AKT-mTOR signaling pathway activation (figure 1). The regulatory effect of LIF on breast cancer metastasis has been determined, using *in vivo* lung metastatic assays and *in vitro* trans-well assays. T47D and MDA-MB-231 cells treated by LIF were injected into mice and in result enhanced lung metastasis and induced distant metastasis to the neck, back, and muscle. The role of LIF on breast cancer metastasis is independent of estrogen receptor status; LIF has a similar effect not only on ER-positive breast cancer cells, including MCF7 and T47D but also on ER-negative breast cancer cells like MDA-MB-231 (Li *et al.* 2014). The mechanism by which the LIF has promoted metastasis of breast cancer is the activation of the mTOR signaling pathway through AKT. The mTOR activation has suppressed by inhibiting AKT and in result has inhibited metastasis of breast cancer.

Previous studies have reported that LIF production was induced by TGF- β in cancer-associated fibroblasts leads to pro-invasive activation of fibroblasts and raises carcinoma cell invasion (Albrengues *et al.* 2014). *In*
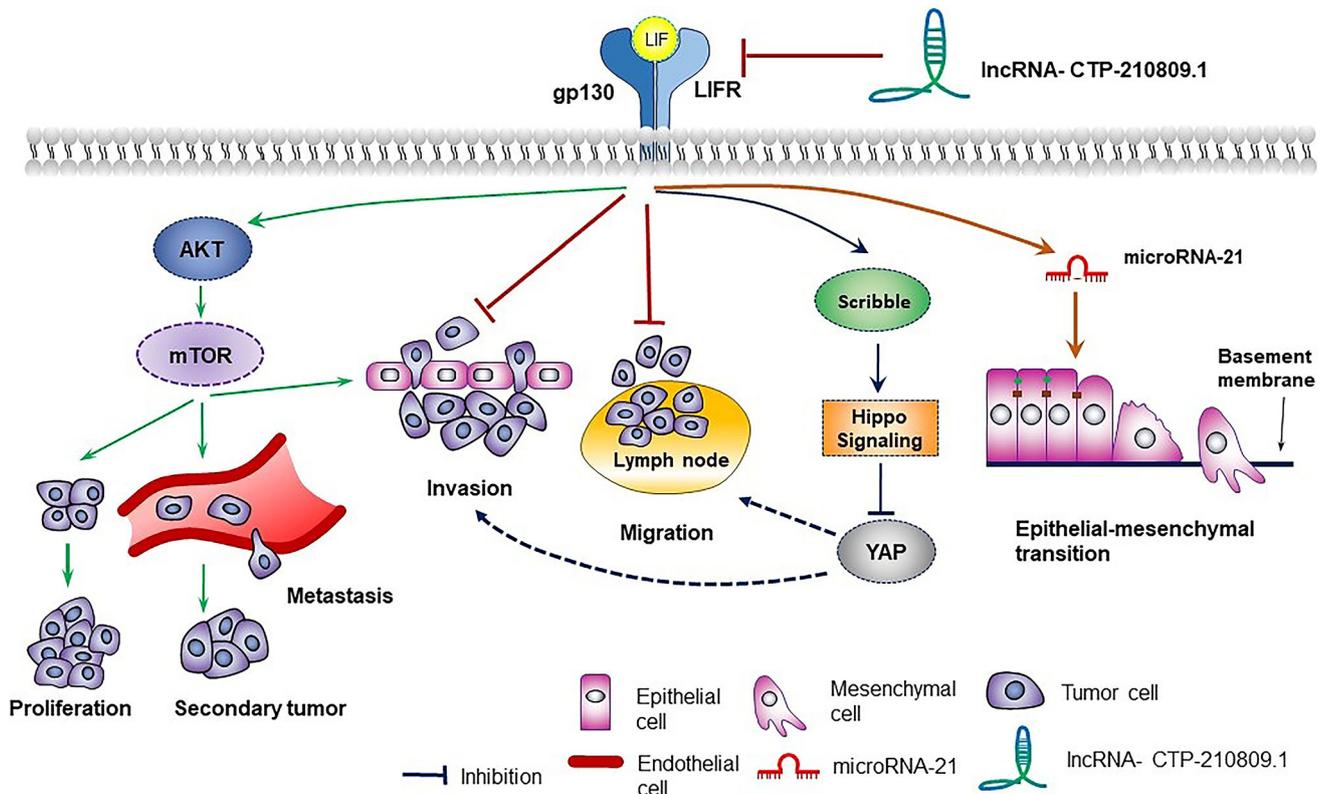
Figure 1. Functional role of LIF in breast cancer. LIF increased the breast cancer cells proliferation, invasion and metastasis through AKT-mTOR signaling pathway activation. LncRNA-CTD-2108O9.1 inhibits migration and invasion of breast cancer cells through a LIFR-dependent pathway. LIFR alters localization of Scribble and induces activation of Hippo signaling results in phosphorylation-dependent inactivation of the YAP and LIFR suppress metastasis of breast cancer. LIF promotes expression of miR-21 through the STAT3 activation and miR-21 induces EMT.

vivo study showed long noncoding RNA-CTD-2108O9.1 could target LIFR and suppress metastasis of breast cancer through a LIFR-dependent pathway. LncRNACTD- 2108O9.1 has low expression in breast cancer tissues and cell lines, and high expression in normal breast epithelial cells. Lower expression of lncRNA-CTD-2108O9.1 was correlated with high metastasis to lymph node and *in vitro* studies showed overexpression of lncRNA-CTD-2108O9.1 prevents breast cancer cell migration and invasion (figure 1) (Wang *et al.* 2018).

Metastasis of highly malignant tumor cells suppresses by restoring the LIFR expression in these cells. LIFR alters the localization of Scribble (an upstream regulator of Hippo signaling and is an assembled adaptor of a protein complex) and activates Hippo signaling, a tumor suppressor cascade, leading to phosphorylation-dependent dysregulation of the transcriptional co-activator YES-associated protein (YAP) (Fig.1). In contrast, migration and invasion of non-metastatic breast cancer cells, induced by loss of LIFR through activation of YAP. Therefore, LIFR is a metastasis suppressor of breast cancer (Chen *et al.* 2012). Patients with LIFR-negative breast tumors, showed high distant metastasis with poor prognosis (Piccolo 2012). Upregulation of miR-9, an upstream regulator of LIFR and E-cadherin, results in inhibition of membrane localization of Scribble and Hippo kinases in E-cadherin-negative/LIFR-negative tumor cells. This leads to YAP nuclear repletion and induces metastasis (Ma *et al.* 2010). It has been proposed that LIFR suppresses the metastasis by opposing the YAP activity (Piccolo 2012).

4.4 *LIF promotes Epithelial-mesenchymal transition*

The LIF overexpression promotes the development of mesenchymal features in tumor cells; leads to epithelial-mesenchymal transition (EMT) in breast tumor cells; reduces epithelial marker E-cadherin expression at mRNA and protein level, and enhances mesenchymal markers such as vimentin and N-cadherin (Yue

et al. 2016). EMT has a significant function in tumor metastasis. Endogenous LIF knockdown, using two different shRNA vectors, reverses EMT in breast cancer cells. Overexpression of miR-21through the activation of STAT3 at the down-stream of the LIF/LIFR pathway induces EMT by decreasing E-cadherin and increasing Vimentin and N-cadherin expression (Fig. 1). Additionally, miR-21 affects several genes (e.g. *PTEN*, *TIAM1*, *PDCD4* and *maspin*) that are inhibitors of migration, invasion and metastasis (Asangani *et al.* 2008; Cottonham *et al.* 2010). The above findings indicate that LIF promotes cancer metastasis through EMT of tumor cells. Interestingly, blocking of miR-21 suppresses the LIF effect on morphological variations of cells from epithelial to mesenchymal and consequently suppresses breast cancer cell migration (Yue *et al.* 2016).

4.5   *LIFR is a breast tumor suppressor*

The whole-genome human RNAi library is used to recognize functional tumor suppressor genes. Based on this collection, LIFR has been established as a tumor suppressor that its deregulation may contribute to the transformation of a large number of human breast cancers (Iorns *et al.* 2012). LIFR transcription expression is greatly reduced in breast carcinoma, hepatocellular carcinoma and colon adenocarcinoma compared to normal samples and is inversely correlated with tumor grade. The finding provides evidence that during breast tumorigenesis, loss of LIFR expression occurs and indicates that LIFR may be a clinically important breast tumor suppressor. HMLERs cells transformed the non-tumorigenic cell line, treated with 25 ng/mL of LIF, induced P-STAT signaling pathway and decreased the invasion and migration. This study concluded that LIFR suppresses breast cancer cell invasion and migration (Dempsey *et al.* 2016).

4.6   *LIF stimulates activation of fibroblasts to promote invasiveness*

Growth factors and cytokines secreted by the cancer cells within the tumor microenvironment activate the adjacent fibroblasts (Calvo and Sahai 2011; Phan 2008). *In vitro* experiment showed that LIF secretion was high in invasive breast carcinoma cells and LIF production was low in noninvasive cancer cells. Mouse tumor cells produced LIF that contributed to the contractility of mouse fibroblasts (Albrengues *et al.* 2014). It has been reported that thirty days after injection of different type of breast carcinoma cells, which produce LIF in low and high levels, into mammary fat pads of syngeneic BALB/c female mice, high LIF secretion was particularly reported in the primary tumor mass that produced by LIF high-producer cells and this is associated with activation of STAT3 in fibroblasts (Albrengues *et al.* 2014). It has been shown that conditioned media (CM) of LIF high-producer cells stimulated mouse dermal fibroblasts and leads to activation of SMAD2 and expression of alpha-smooth muscle actin (α-SMA), a hallmark of cancer-associated fibroblasts. Whereas CM of LIF low-producer cells failed to stimulate α-SMA expression and activate SMAD2 and STAT3. They identified that LIF mediates the pro-invasive activation of stromal fibroblasts independent of α-SMA expression (Albrengues *et al.* 2014).

4.7   *LIF-mediated signaling in breast cancer*

LIF-LIFR complex activates the LIF signaling pathway, including JAK/STAT3, PI3K/AKT, MAPK, and/or ERK1/2 in different tissues and cell types (Gearing *et al.* 1992; Gearing *et al.* 1991). Li *et al.* demonstrated that mTOR signaling was activated by LIF in breast cancer (Li *et al.* 2014). The mTOR pathway activation in breast cancer is a significant contributor to tumor development and metastasis (Seeliger *et al.* 2007; Wander *et al.* 2013). Inhibition of the mTOR pathway revoked the metastasis effect of LIF in breast cancer, therefore, mTOR pathway activation intervenes with the enhancing tumorigenesis and metastasis effect of LIF in breast cancer (Li *et al.* 2014). The other study showed that AKT interferes with LIF activated mTOR pathway (Ohbayashi *et al.* 2007; Slaets *et al.* 2008). AKT inhibition, using wortmannin greatly abolishes the mTOR activation and abrogates enhancing the effect of LIF on breast cancer tumorigenesis and metastasis. Hence, LIF through the AKT-mTOR signaling pathway enhances tumorigenesis and metastasis (Li *et al.* 2014).

The high level of LIF expression and activated STAT3 have been identified in mouse breast tumors and their primary cultures (Quaglino *et al.* 2007). This observation suggests the impact of LIF on the STAT3 activation in mouse mammary tumors, leading to an increase in tumor cell viability. After LIF-blocking, using an antibody, STAT3 phosphorylation was suppressed (Quaglino *et al.* 2007). MiRNA-125a affects the LIF receptor and results in the homeostasis of not only the nonmalignant but also malignant breast epithelial stem cells via the Hippo signaling pathway. Activation and suppression of LIFR are associated with

the suppression and high expression of miR-125a, respectively (Nandy *et al.* 2015). Activation of the Hippo signaling pathway inactivates YAP protein and results in the inhibition of migration and invasion.

4.8 *Therapeutic approaches involving LIF-LIFR signaling*

It has been reported that LIFR-JAK1-STAT3 signaling limits the efficacy of histone deacetylase (HDAC) inhibitor, an epigenetic-based cancer therapy, on breast cancer (Zeng *et al.* 2016). HDACi upregulates LIFR and results in activation of JAK1-STAT3 signaling and reduces drug response in breast cancer. HDACi enhances histone acetylation at the LIFR gene promoter and upregulates LIFR expression through recruiting bromodomain protein BRD4 and consequently regulates JAK1-STAT3 signaling and restricts the reaction to HDAC inhibition. It has been shown that simultaneous inhibition of BRD4 or JAK increases the effect of HDAC inhibitors in triple-negative breast cancers. These results represent that inhibition of LIFR-JAK1-STAT3 signaling by BRD4 and JAK inhibitors could be a potential therapy for breast cancer (Zeng *et al.* 2016). In contrast, evaluation of ruxolitinib, a selective JAK1/2 inhibitor in the LIF-JAK-STAT3 signaling pathway, has shown that anti-tumor efficacy was inhibited in metastatic triple-negative breast cancer patients despite evidence of on-target activity (Stover *et al.* 2018). It has been suggested that this could be because of incomplete inhibition of JAK-STAT by ruxolitinib or a cytostatic rather than a cytotoxic effect. Furthermore, intratumoral heterogeneity may interfere with resistance (Stover *et al.* 2018).

The LIF/LIFR pathway is involved in breast cancer metastasis through activation of the mTOR pathway. Li *et al.* demonstrated that rapamycin, a specific inhibitor of mTOR, is suppressed the effect of LIF in the invasion and migration of MCF- 7, T47D and MDA-MB-231 cells (Li *et al.* 2014). Taken together, these results suggest that targeting LIF/LIFR signaling might be a potent therapeutic strategy for breast cancer and the prevention of tumor recurrence.

5. Conclusion

The results presented here indicate that the LIF and LIFR expression were observed in 80% of breast tumors. LIF expressed in several human breast cancer cell lines, such as T47D, MCF7, SK-Br-3, HS578T, MDA-MB-232, BT474, and MDA-MB-468 cells, malignant, nonmalignant and one normal breast cell line. The LIF expression level correlated with cell line metastatic ability and was higher in cells with high metastatic ability. LIF significantly contributes to the development of growth, tumorigenesis, invasion and metastasis of breast cancer. However, the effect of LIF on normal breast epithelial lines was less significant, leading to inhibition of the proliferation. The effect of LIF on breast cancer is regulated by JAK/STAT3, PI3K/AKT and AKT-mTOR pathways. The LIF expression enhanced the proliferation through activation of the AKT-mTOR signaling pathway in breast cancer cells. The knockdown of endogenous LIF decreased breast cancer cell growth and inhibited the mTOR signaling and tumorigenesis. In addition, LIF increased invasion and metastasis of breast cancer cells through AKT-mTOR signaling pathway activation. Metastasis of malignant tumor cells suppressed by the LIFR expression in these cells. LIFR regulates Scribble and leads to activation of Hippo signaling through YES-associated protein. LIF has a direct potential to develop EMT in breast tumor cells and decrease the expression of epithelial markers and increase mesenchymal markers. LIF developed the expression of miR-21 through the STAT3 activation and this miR-21 induces EMT. LIF activates STAT3 in mouse mammary tumors and enhances tumor cell viability. Taken together, this study strongly suggests that LIF is an important prognostic biomarker for breast cancer and can be a therapeutic target for clinical intervention.

6. Future perspective

Further investigations of the effects of LIF as a therapeutic target for breast cancer and the means of interrupting its stimulatory pathways are warranted.

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