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REVIEW PAPER

Decoding Insulin-Like Growth Factor Signaling Pathway From a Non-coding RNAs Perspective: A Step Towards Precision Oncology in Breast Cancer

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Abstract

Breast cancer (BC) is a highly complex and heterogenous disease. Several oncogenic signaling pathways drive BC oncogenic activity, thus hindering scientists to unravel the exact molecular pathogenesis of such multifaceted disease. This highlights the urgent need to find a key regulator that tunes up such intertwined oncogenic drivers to trim the malignant transformation process within the breast tissue. The Insulin-like growth factor (IGF) signaling pathway is a tenacious axis that is heavily intertwined with BC where it modulates the amplitude and activity of vital downstream oncogenic signaling pathways. Yet, the complexity of the pathway and the interactions driven by its different members seem to aggravate its oncogenicity and hinder its target-ability. In this review, the authors shed the light on the stubbornness of the IGF signaling pathway and its potential regulation by non-coding RNAs in different BC subtypes. Nonetheless, this review also spots light on the possible transport systems available for efficient delivery of non-coding RNAs to their respective targets to reach a personalized treatment code for BC patients.

Keywords Insulin-like Growth Factor · IGF1R · Breast Cancer · MicroRNAs · Long non-coding RNAs · Combination Therapy · Precision Oncology

Introduction

Breast Cancer (BC)

Ever since breast cancer (BC) was first discovered by ancient Egyptians [1], there has been a continuous rise in BC cases worldwide. The number of cases identified in 2020 reached approximately 2.3 million cases making it the most diagnosed type of cancer in 2020 [2]. Women are the main target sector for the disease, BC prevails in developed countries, yet the mortality rates of developing countries are much higher [2]. BC has been suggested to prevail in women in their 40–59 age period, together with other risk factors and lifestyle traits that also contribute to the disease development [3].

Heterogeneous Nature of the Disease: A Step towards Precision Oncology

BC tumors lack compositional uniformity [4]. In other words, the heterogeneity displayed by BC is the reason behind BC's complexity and represents a huge challenge that has to be resolved to gain a better understanding of the disease [4]. After several investigations, six major BC molecular subtypes were identified (Table 1) [5–7]. BC subtypes largely differ in their molecular characteristics, incidence rate, prevalence, diagnosis, prognosis, metastatic rates, and treatment approaches as summarized in Table 1. These classifications aided in better resolution of the disease yet other molecular differences between individuals are also important parameters when it comes to the main concept of precision medicine [1]. Identifying potential therapeutic targets

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Table 1 Major six Breast Cancer Subtypes

	Luminal A	Luminal B	HER2 positive	Basal-like (triple-negative)	Normal-like	Claudin- low
Gene expression	ER + PR + HER2- Ki-67 low	ER + PR + HER2-/+ Ki-67 high	ER- PR- HER2 +	ER- PR- HER2- But shows cytokeratin (CK516, CK14) increase	Markers of EMT, immune response and SCs	ER- PR- HER2-
Prognosis	Most favorable	Worse prognosis than luminal-A	Poor prognosis	Worst prognosis	Intermediate prognosis between luminal and basal-like	Worst prognosis
Incidence	Most common (50–60%)	15–20%	15–20%	8–37%	5–10%	Less common
Aggressiveness	Least aggressive	More aggressive than luminal-A	More aggressive	Most aggressive	Slightly more aggressive than luminal A	aggressive
Metastasis	Bones (58.52%) Brain (4.30%) Liver (15.48%) Lung (21.70%)	HER2-: Bones (58.52%) Brain (4.30%) Liver (15.48%) Lung (21.70%) HER2 + Bones (47.28%) Brain (5.89%) Liver (25.65%) Lung (21.17%)	Brain (34.49%) liver (31.72%) Bones (34.49%) Lung (25.48%)	Brain (9.12%) Lungs (32.09%) Bones (36.39%) Liver (22.40%)	Add the most common metastatic site	Lung metastasis
Treatment	Hormonal therapy only or might require the addition of chemotherapeutic agents	A combination therapy containing Hormonal therapy and chemotherapy (and anti-HER2 therapy if HER2 positive)	Anti HER2 targeted therapy (Trastuzumab, Lapatinib, gefitinib, neratinib, erlotinib, and afatinib) and Chemotherapy	Targeted therapy	Chemotherapy and/or targeted therapy	Targeted therapy
Characteristics	Low histological grade, low cellular proliferation, and high nuclear resemblance	Higher histological grade, higher cellular activity, and lower survival rates after relapse compared to luminal-A	High cellular division,75% have a high histological grade and nuclear pleomorphism	High proliferative activity, high histological grade, and low survival rates	Shows pattern similar to normal breast tissue	Has triple-negative tumors with epithelial-to-mesenchymal transition features, immune response, and cancer stem cell-like markers
Prevalence	23.7%	38.8% (HER negative) 14& (HER positive)	11.2%	12.3%	7.8%	N/A

through analyzing malignant signaling cascades fueling BC hallmarks has been considered a vital step towards precision oncology [8].

Oncogenic Circuits Fueling BC Tumorigenicity

The physiological mammary development within the breast tissue is a complex process associated with several cascades that may induce tumorigenesis [9]. For instance, the estrogen and HER2 receptors, which are normally activated during the physiological development of the mammary glands are the same receptors associated with breast tissue malignancy and driving BC stem cells [10–14]. Some of the most common deregulated axes in BC are the JAK/STAT and P13K/AKT/mTOR pathways where the overexpression of any of the members linked to these axes is considered a red flag indicating resistance to hormonal therapy and more aggressive BC phenotypes [15, 16]. The inflammatory response of cells at the tumor microenvironment (TME) was also found to be highly related to the activation of the aforementioned oncogenic signaling pathways [17]. It is worth mentioning that such highly prevalent oncogenic signaling pathways are drawn downstream receptor tyrosine kinases (RTKs) in several malignant contexts including BC [15, 16, 18].

Insulin-like Growth Factor-1 Receptor (IGF1R): A Key Player Regulating Oncogenic Circuits

Insulin-like growth factor 1 receptor (IGF1R) is one of the most resistant RTKs that is associated with several oncogenic signaling pathways as shown in Fig. 1. IGF1R has been reported to play a key role in modulating several oncogenic signaling pathways such as JAK/STAT, PI3K/AKT/mTOR, and RAS/RAF axes [19–23]. Each of the aforementioned axes is solely a potent promoter of BC progression and epithelial-mesenchymal transition (EMT) [24]. The association of IGF1R in BC has been extensively studied in terms of inducing malignancy, as well as, normal mammary development [25, 26]. Its role in inducing resistance to conventional therapeutic approaches among BC patients was also noted [27, 28]. On the other hand, several clinical trials showed that inhibition of IGF1R via different methods did not efficiently shut down IGF1R downstream signaling pathways, a matter that highlights the complexity of the IGF signaling pathway and spots the

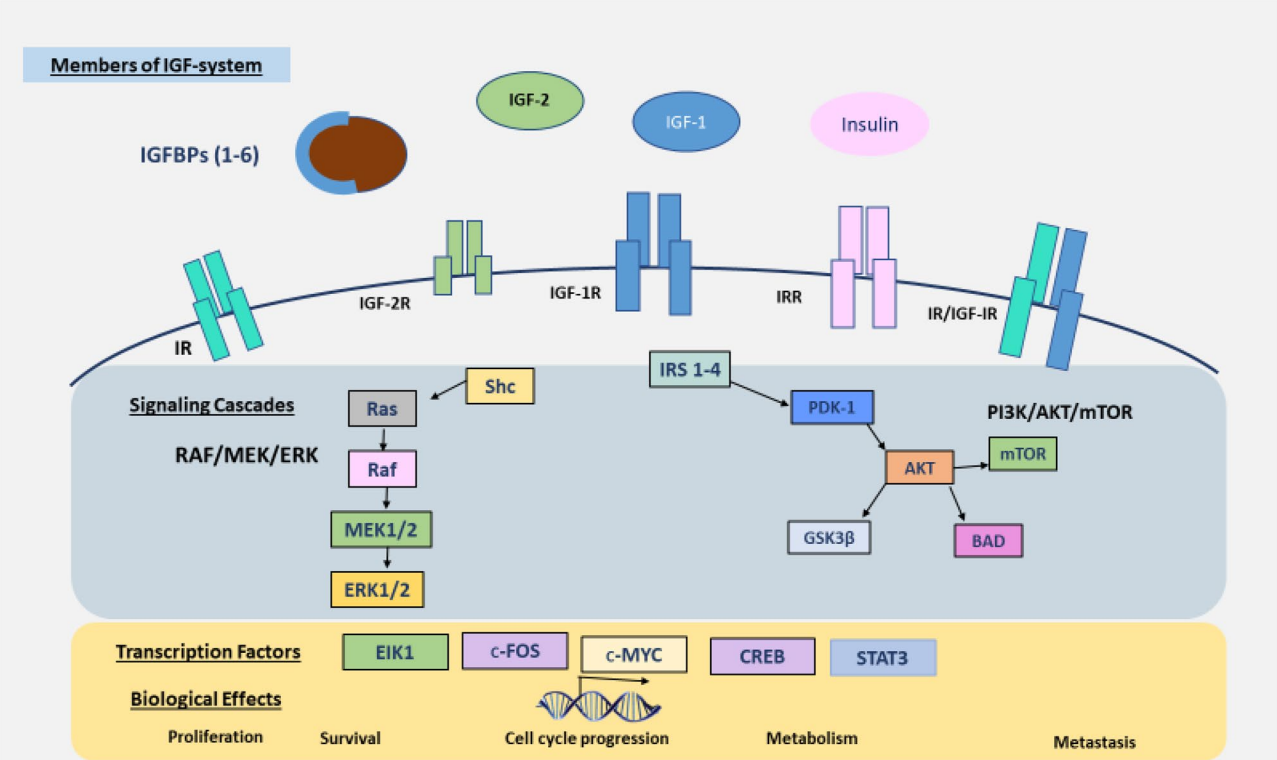

Fig. 1 Representative figure for Members of the IGF signaling pathway and its associated oncogenic Signaling pathways. IGF signaling members mainly comprise ligands (Insulin, IGF1, and IGF2), Receptors (IR, IGF2R, IGF1R, IRR, IR/IGF1R hybrid), and IGFBPs (1–6). Signaling cascades initiated mainly through the gatekeeper of the axis (IGF1R) activation are RAF/MEK/ERK pathway, JAK/STAT pathway, and PI3K/AKT/mTOR pathway.

light onto the urgent need to search for efficient upstream regulators for the axis to properly modulate it [29, 30].

IGF1R: A Dominating Receptor in BC

IGF1R and the IGF axis, in general, have been extensively reviewed as dominant players in BC. For instance, IGF1R activation is a fundamental fuel for breast cancer stem cells as it is essential for inducing BC cellular viability and aggressiveness [24].

As previously mentioned, although several research groups are working on extensive investigative trials related to IGF1R signaling, the development of effective anti-IGF1R molecules is still under investigation [27, 31].

The huge potential of non-coding RNAs (ncRNAs) in regulating tumorigenesis is currently considered a research hotspot. Along with this, the disappointing results of the clinical trials for therapeutic agents targeting the IGF signaling pathway further emphasize the need to link the possible modulatory effects of ncRNAs to IGF members, which will be immensely investigated in this review.

The Significance of the Insulin-like Growth Factor (IGF) Axis in BC

The alarming effects of IGF1R activation by either insulin or insulin-like growth factor 1 (IGF1) has been an attractive ongoing research area due to the huge contribution demonstrated by the IGF axis in normal mammary development and therefore induction of BC [32]. Deep investigation of IGF members further showed the complexity of this signaling pathway where several cross-talks between various members of the pathway and other receptors have been reported in BC [33]. Moreover, the indisputable role of the IGF pathway in chemo-resistance and involvement in the metastatic nature of BC has increased the alertness to this regulatory circuit [32]. Failure to develop functional therapeutic agents for proper inhibition of the tumorigenic effects induced by the IGF signaling pathway gives rise to an important research gap that has to be fulfilled [32]. Likewise, the intricate crosstalk between the IGF signaling pathway and other oncogenic axes provides it with the ability to activate other parallel or downstream signaling pathways when any member of the IGF axis is therapeutically targeted. Accordingly, this review tackles the nature, physiological and pathological roles of IGF family members as vital players in BC initiation, progression, and metastasis as presented in Table 2 and their possible regulators.

Dissecting the IGF Members

The Story Behind IGF Ligands Nomenclature

The odd discovery of the IGFs has generously contributed to the scientific world, the ligands were first discovered as sulphation factors produced by growth hormone (GH) in rats lacking the hypophysis or the pituitary glands where these ligands were able to stimulate sulfate incorporation in cartilaginous tissues [34]. Their actions then evolved showing non-suppressible insulin-like activity and they were later referred to as somatomedins due to their conciliation effects on GH [34]. Finally, conclusions regarding their structure and functions linked them to proinsulin and insulin, resulting in naming them as insulin "like" growth factors 1 and 2; 'IGF1 and IGF2' [35]. These belong to a family of hormones that comprise four major metabolically significant single chains of polypeptides; insulin, proinsulin, IGF1, and IGF2 [36]. Both IGF1 and IGF2 ligands are present in human sera throughout different stages of the lifecycle. The main difference between both ligands lies in their regulatory entities: IGF1 is mainly affected by GH or other transcription factors found in local tissues and is predominantly found in high concentrations throughout childhood. On the other hand, IGF2 is mainly expressed in prenatal stages and is not controlled by GH secretion rather, the genomic imprinting of the gene itself [36].

A Snapshot On the IGF Regulated Molecular Signaling Circuit

The normal cell to cell signaling processes that are initiated by IGF binding and the subsequent auto-phosphorylation of the IGF1R further activate phosphorylation cascades through binding specific substrates as the insulin receptor substrates (IRS) 1–4, Src homology 2 domain-containing (SHC) which are the key players in activating other downstream effectors that are responsible for the functional activities orchestrated by this axis. The PI3K-AKT pathway activated by the IRS has multiple metabolic, migrative, and proliferative effects due to activation of mammalian target of rapamycin complex I (mTORC1) and GSK3 as presented in Fig. 1. This cascade also promotes cell survival where AKT independently induces antiapoptotic function through inhibition of B-cell CLL/lymphoma 2 antagonists of cell death protein responsible for cell death. Meanwhile, the RAS-RAF and MAPK pathways phosphorylation due to SHC binding predominantly occurs by IGF2 rather than the IGF1 initial binding mediating the activation of transcription factors prompting their growth-stimulating effects on cells as shown in Fig. 1 [36–38].

Table 2 Nature, physiological functions, the pathological role of IGF axis members in Breast Cancer

Member	*Nature*	*Physiological role*	*Pathological role in BC*	*Prognostic value*
IGF1	Growth hormone is a small single-chain polypeptide made up of 70 amino acids Encoded by chromosome 12	Stimulating cellular proliferation and differentiation, thus ensuring proper growth and development Neurogenerative, and hepatic protectant Anti-oxidant Necessary for normal mammary development	IGF1R induced proliferation and anti-apoptotic activity	High plasma levels are associated with poor prognosis in ER + BC
IGF2	Structurally homologous to IGF1 (75%) Difference in one amino acid might be the reason for IGF-2R binding	Necessary for correct fetal growth and development Responsible for cellular differentiation in specific tissues	IGF1R induced proliferation and anti-apoptotic activity IR-A induced mitogenic activity	Elevated IGF2 by tumors is of poor prognostic value
IGFBPs (1–6)	Binding proteins comprised of 216–289 amino acids with structural homology in N- & C-terminals and different linker domains	A variety of functions by the different subgroups including their roles in metabolism, fetal and bone growth Inhibit IGF actions May potentiate other non-IGF members, these actions are not related to the IGF axis	The most common is Inhibiting IGF-induced mitogenic and tumorigenic pathways Different members have different effects on BC progression, some have shown tumor-promoting activity instead	Some are of prognostic values IGFBP4-5 expression is of favorable prognosis in ER + BC IGFBP5 expression is of poor prognosis in ER-negative BC Other IGFBP's prognostic values are debatable
IG1-R	TKR, 1367 amino acids TK domain Juxtamembrane domain Distinctive C-terminus Located on chromosome 15 Two alpha & Two beta subunits form a heterotetramer	Necessary for normal growth and development Mediation of metabolic pathways	Acts through several tumorigenic pathways promoting the mitogenic activity of cells, stemness in the EMT, and metastasis Can bind to DNA and regulate gene transcription Associated with BC resistance to therapy	Varies through literature where its expression has been associated with poor prognosis in BC generally, or in ER + BC specifically or it may even have a positive prognostic value in luminal BC
IGF2-R	Chromosome 6q No TK domain	necessary for proper growth	Tumor suppressor Important for controlling the bioavailability of IGFBP2	-
IGF2BPs	Previously mentioned Has a distinctive triamino acid sequence in its C-terminal potentiating integrin-binding	Regulates IGF activity in CNS Regulate metabolism and adiposity	IGF-independent actions, enhances cellular proliferation, anti-apoptotic activity, and invasiveness Reduces PTEN: tumor suppressor	High circulating levels of the binding protein are linked to aggressiveness, poor prognosis

Regulatory Mechanisms Conciliating IGF1R Expression

IGF1R regulation is necessary to avoid all the repercussions associated with the receptor's hyperactivation [39]. Normally, IGF1R expression is tightly regulated by protein tyrosine phosphatase 1B (PTP1B), a protein that inhibits the autophosphorylation of the receptor via binding to the β-subunit of the IGF1R [40]. The depletion of PTP1B in mammalian cells leads to increased phosphorylation and tyrosine kinase activity indicating that PTB1B might be essential for the negative regulation of the IGF1R [40]. In other words, IGF1R's downstream effects are enhanced in absence of PTP1B regulation due to an increase in AKT and ERK phosphorylation, which further enhance the cellular growth rate and transformative ability, as well as, anti-apoptotic activity and motility [40, 41]. However, throughout the years, it was found that the expression of PTP1B varies for different types of cancers. For instance, Fan et al. found that PTP1B was underexpressed in ovarian cancer cells, moreover, the restoration of PTP1B expression increased cellular apoptotic activity and decreased cellular proliferation. [42]. Conversely, several others have found that high PTP1B levels are characteristic for some BC subtypes where as a result of this overexpression, increased ERK activation was observed, as well as, enhanced cellular migration induced by IGF2, enhancing the metastatic potential of BC [43]. In HER2 BC, PTP1B overexpression in breast tissue was also observed and linked to mediating breast transformation, surprisingly, the depletion of PTP1B reduces BC hallmarks and also decreases the risk of metastasis [44, 45]. Consequently, PTP1B inhibitors seem to be valuable for HER2 cancers overexpressing PTP1B [46, 47]. Similar effects were also observed in TNBC giving rise to the need to develop inhibitors for PTP1B or find key regulators that control its expression [48].

Another important regulator of IGF1R activity is the SHP family of proteins where SHPS1 induces the binding of SHP2 to the IGF1R, which results in dephosphorylation of the receptor after the initial activation of the IGF1R. This regulatory network is necessary to ensure IGF1R is inactivated post IGF1 binding [49]. On the contrary, Li et al. revealed another layer of complexity to tyrosine phosphatase regulation of the IGF1R where SHP2 was found to be overexpressed in BC tumors, this overexpression seems to be favorable for ER and IGF1R activation along with, ERK and AKT phosphorylation [50]. Additionally, knockout of SHP2 retarded mammary tumorigenesis in mice [50]. Elevated SHP2 expression in BC was also recently linked to poor prognosis in BC patients, the exact mechanism by which SHP2 facilitated cellular and tumor growth is through manipulation of cyclin D and P13K/AKT signaling [51].

Physiological Role of IGF Ligands

The continuous unraveling of IGFs' functions has been taking place for decades highlighting the magnitude of their physiological roles in the human body. Nonetheless, the turbulence that occurs when the IGF ligand is deficient further displays the importance of the growth-promoting roles of the IGF ligands and their association with GH as a modulator [36]. The functions of IGF are numerous and vary from one tissue to the other; yet the most prevailing is the ability of the ligand to promote cellular proliferation and differentiation [36, 52]. The various functions of the ligand in healthy individuals contribute to proper growth and development, this can occur in different cell types such as chondrocytes, smooth muscles, and most importantly, luminal and myoepithelial cells of the breast [53–55]. Moreover, it has been reported that IGF1 working in a paracrine/autocrine manner showed an indisputable role in mediating GH-induced somatic growth as validated by knockout mice models and several other studies. The ability of IGF1 to stimulate DNA synthesis in the mammary gland was a huge revelation that intrigued further analysis and investigations [56]. Henceforth, the role of IGF1 in females is prominent where additional functions of the ligand are required for pubertal growth, pregnancy, and milk secretion [33, 57–59]. The synergism between the ligand and hormones is essential for the correct mediation of these processes [33]. Other functions for the ligand also exist, these include neurological and hepatic protection, additionally, the ligand's contribution to follicular development and its unique rejuvenating properties and anti-oxidant abilities have made it of increasing popularity and a huge discovery to the science world [38, 58, 60–64].

Pathological Role of IGF Ligands: A Special Emphasis On BC

The upregulation of the IGF ligands or the hyperactivation of IGF receptors aid in breast tissue's malignant transformation process. This is because ligands of the IGF family are extremely homologous, meaning that IGF ligands can bind to IGF1R, insulin receptor (IR), and most importantly IR-IGF1R hybrids, which are highly related to the initiation of malignancy signals [24]. This further provides the ligands with the ability to regulate several cellular metabolic processes through stimulation of the IR, which may positively contribute to BC progression if altered.

IGF axis is also a big contributor to metastatic play. It has been well documented that IGF ligands have an indisputable role in initiating metastasis among BC patients and increasing the aggressiveness of the disease documented by the elevated levels of IGF ligands in BC tissues compared to its normal counterparts and its direct association with poor clinical outcomes [65]. Moreover, the contribution

of the IGF ligand-receptor interactions in inducing EMT characteristics of breast tumors has been well-documented in several reports [66]. Nevertheless, the opposing cellular growth effects stimulated by the IGFs on cells treated with chemotherapeutic agents have resulted in aggressive resistance phenotypes of such treated malignant cells [24].

BC subtypes are numerous as previously mentioned and methods to combat this disease's resistance to therapy and its metastasizing ability are constantly a big obstacle hindering clinicians from having the anticipated clinical outcomes, they expect from the treatment protocol [24, 67, 68]. So here in this review, we shed the light on the IGF signaling pathway which could be a hidden reason behind such poor clinical outcomes.

Clinical Significance of the IGF Ligands Among BC Patients

The prognostic and diagnostic role of the IGF ligands is highly complex due to its dependence on various aspects such as the molecular subtype of BC and the different signaling mechanisms the ligands are capable of performing [68]. High serum IGF2 levels have been generally linked to the risk of development BC, making it a feature of most BC patients. The prognostic role of the ligand was surprisingly promising in BC subtypes except for hormone receptor-positive BC as summarized in Table 2 [69]. Further investigations are still needed to reach elaborative conclusions regarding the presence of IGFs in different BC subtypes and the overall survival of BC patients and thus evaluating its prognostic and diagnostic values among BC patients [69].

IGF Ligands As Therapeutic Targets for Cancer Patients

IGF ligands have been always cast as promising therapeutic targets for several oncological disorders including BC. Repression of IGF ligands will accordingly lower IGF1R induction and anti-apoptotic activity. Such repression takes place by two methods: 1) Inhibition of GH or any regulatory step in the pathway will be sufficient in inducing a reduction in IGF1 levels or 2) Using monoclonal antibodies directly targeting IGF-ligands [67]. Pasireotide is a growth hormone-releasing hormone inhibitor, which has shown favorable outcomes in reducing IGF1 levels via repression of AKT signaling and is currently undergoing phase II clinical trials. JV-1–36 or JMR-132 are another two drugs targeting the same target and have shown anti-tumor activity in the murine TNBC model [67]. IGFs specific monoclonal antibodies have also been implemented as mentioned earlier aiming to abolish IGF1R and IR-B activity without affecting the normal insulin needs of the body, this approach has the advantage of targeting both ligands and therefore their respective receptors, moreover maintaining the normal metabolic environment of cells by leaving insulin out of the equation [67]. MEDI-57R and BI 836,845 are two examples of phase I clinical trial drugs that decrease IGF levels by binding to the ligands instead, lowering their bioavailability and their negative proliferative effects on malignant cells. BI 836,845 has the extra advantage of inhibiting the oncogenic AKT pathway [31, 67, 68, 70]

Unraveling the Structure and Function of the IGF Receptors

As both ligands share some features with insulin, the same concept applies to the receptors where certain domains have a significant resemblance percentage that reaches 80–90% in the tyrosine kinase (TK) domain responsible for the intracellular catalysis of ATP and therefore the auto-phosphorylation [71]. Another two domains are also present in the IGF1R; the juxta-membrane domain responsible for the receptor-mediated endocytosis due to the presence of NPYX motif and finally, the C-terminus necessary for initiation of signaling cascades by IGF1 and to a lesser extent IGF2 binding [71]. The C-terminus of the IGF1R is also responsible for mediating cellular processes governing the cell's transformative ability which is a key complication linked to the hyperactivation of the receptor [72]. All these domains are present in the two β-subunits of the receptor whereas, another two α-subunits are present extracellularly which initiate the first step of IGF1 and IGF2 binding, the different subunits are linked together by disulfide bonds forming a hetero-tetramer structure [73]. The genomic analysis of IGF1R shows that the receptor comprises 1367 amino acids and is located on chromosome 15, contains 21 exons extending through more than 100 kilobases [70]. On the other hand, IGF2R spreads through 130 kilobases and is composed of 48 exons found on chromosome 6q, the significant structural difference between both receptors lies in the missing tyrosine kinase domain from the IGF2R [73]. Nevertheless, the receptor molecule is composed of four distinct regions; the extracellular domain contains the highest number of residues where amino acids 1508–1566 are accountable for IGF2 binding [73]. The other three regions are composed of a lower number of residues but are still important for efficient receptor functioning which are the C-terminus end, the transmembrane region, and the transit peptide at the N-terminus. The IGF2R is known to bind to multiple ligands, most importantly mannose 6-phosphate which contains lysosomal enzymes that break down the IGF2 once it's endocytosed by the receptor, providing regulation for IGF2's levels present in human serum [73]. IGF2R is activated upon binding of IGF2 and to a lower extent IGF1. IGF2R activation can similarly lead to growth stimulation where sphingosine kinase activation stimulates the extracellular release of sphingosine 1-phosphate,

prompting the binding of the ligand to its specific G-protein coupled receptor, which then activates an intracellular signaling cascade that ends with EKR phosphorylation and therefore growth-related effects [38].

Physiological Role of IGF Receptors

IGF1Rs represent a fundamental part of the IGF axis. Numerous studies emphasized the necessity of the IGF1Rs in normal physiological growth and developmental processes as summarized in Table 2. For instance, IGF1R has shown an elementary role in normal lung development as validated by several studies of IGF1R knockout mice [74–76]. Being the point of initiation or activation of growth-promoting signaling pathways adds to its significant regulatory function [36]. The subsequent events and pathways activated following IGF1R activation are also required for the normal metabolic processes of cells due to AKT activation [38].

Pathological Role of IGF1R: A Special Emphasis On BC

The role of IGF1R in BC aggregates controversy between researchers. This goes back to the fact that silencing of the IGF1R did not provide the expected negative impacts on BC hallmarks separate from the side effects of the treatment [29, 77]. Several other studies also show a beneficial side to the receptor where the loss of IGF1R signaling in the Wnt1 mouse model of basal-like BC decreases tumor latency and converts the tumors to becoming metastatic [78, 79]. However, the involvement of the receptor in stimulating the growth and survival of BC cells cannot be denied. The association of IGF1R with inducing growth-promoting pathways is inevitable owing to ERK activation [36]. Nonetheless, the tangled crosstalk between IGF1R and other oncogenic signaling pathways and other RTKs further supports the notion of linking the IGF1R to undesired effects in BC [80]. For instance, IGF1R expression in breast cancer stem cells is directly associated with the Wnt/β-catenin axis, notch, and hedgehog pathways further providing the receptor with properties related to EMT induction, stemness, and accordingly metastasis [80]. Additionally, the entanglement of the IGF1R to hormone-positive BC has been immensely documented throughout literature where the synergistic effect of estrogen on the IGF1R results in its overexpression and thus the increase in mitogenic activity [81]. This gave rise to several other questions regarding the role of IGF1R in BC cases resistant to endocrine therapy later leading to the conclusion that the receptor interaction with IGF1 might be an alternative pathway in BC cases when estrogen is depleted [81].

In addition, the trastuzumab resistance phenomena further unraveled a new layer of complexity tuning threadbare crosstalk between the IGF1R and HER-2 receptor families. Furthermore, the stubborn nature of tumors resistant to therapy has been highly linked to high transcript levels of IGF1R in tumor cells and its subsequent downstream activation of mTOR [37]. Thus, inhibition of both receptors (IGF1R and HER-2) may be a compulsory approach to reduce tumor growth and AKT activation [37].

Clinical Significance of IGF1R

The dilemma of the prognostic value of the IGF1R in BC patients remains questionable; the expression of the receptor by circulating tumor cells (CTCs) has been surprisingly linked to a better prognosis than CTCs devoid of the receptor's expression [82]. Moreover, a lot of paradoxes concerning the significance of its expression levels in the serum and breast tissues and its correlation with the clinical data and differentiation level of cancer cells [83]. All these contradictions might be due to the differential expression of the IGF1R by BC tumors where the upregulation of the receptor is more predominant in luminal subtypes compared to other non-luminal ones. Such upregulation of IGF1R in luminal subtypes could be explained by the compensation in ER downregulation by IGF1R resistance and the interconnection between both pathways where hormonal regulation may be the main cause of such a phenomenon [24, 67]. Interestingly, such induction in IGF1R levels is more prominent in the aggressive luminal B subtype [24, 67], providing IGF1R as a possible diagnostic marker for the discrimination between luminal A and luminal B subtypes together with Ki-67 levels as shown in Table 2 [24]. The prognostic value of the latter is highly controversial, where the expression of the receptor varies from one BC subtype to another, for instance, estrogen-dependent tumors are considered of poor prognosis if the IGF1R is found to be highly expressed [37, 84, 85]. Yet, others have also shown that IGF1R association with ER predicts a better outcome where its expression is mainly associated with ER + tumors, and its low expression was observed in TNBC [37, 78, 86].

IGF1R As a Promising Therapeutic Target for Cancer Patients

Being a widely discussed topic by scientists over the years, the IGF1R represents the perfect target in this pathway due to its continuous relation in driving the oncogenic profile of cells resistant to therapy; this explains why countless clinical trials take place to develop proper inhibitors of IGF1R [67]. However, the experimental settings of the trials conducted did not always take into account all important factors, for instance, 1) the patients were never tested for IGF1R overexpression in the first place, 2) the use of monoclonal antibodies selective for IGF1R failed to consider the role of insulin receptor and the interconnection between both pathways [67, 69]. For proper development of therapy, the pathways that are linked to IGF1R need to be identified and the mechanisms by which they can

induce resistance need to be properly deducted for the complete inactivation of such a huge pathway, combination therapy is more likely to be used in that case due to the association of multiple receptors and signaling processes. Additionally, finding diagnostic markers for IGF1R overexpression seems necessary due to the contradictory nature of the receptor previously discussed which may go back to the fact that some of the tumors might not even be extensively expressing the receptor.

The connection between the IGF pathway and insulin also serves as a critical parameter that needs to be considered when identifying and developing therapeutic targets and agents respectively [87]. This interlinkage is due to several structural and regulatory factors, which include the resemblance of both receptors [87]. Furthermore, the two types of insulin receptors have different functions in the body where IR-A is mainly expressed during intrauterine life due to its ability to induce growth-promoting signaling cascades yet can also be expressed throughout the years [87]. While IR-B is the predominating form responsible for most metabolic actions induced by insulin in adult life [87]. This explains why overexpression of IR-A is most likely to be associated with carcinogenesis [87]. Therefore, targeting the IGF1R solely may not be sufficient in inhibiting IGF-induced tumor growth activity [87]. Another aspect that also has to be considered when targeting the IGF pathway is the ability of some cells to express receptors of combined dimers, forming INSR and IGF1R hybrid receptors, which also is an important factor to consider upon targeting the IGF1R [87]. In such context, Sentuzumab (BI 836,845) is a monoclonal antibody that showed promising results due to its ability to inhibit both the actions of IGF1R and IR-A [67]. This co-inhibition prompts pro-apoptotic effects on cells without affecting the normal blood glucose level of patients, making it a significant potential agent, currently undergoing phase II trials [67]. Yet, the majority of clinical trials did not represent the expected results upon targeting IGF1R resulting in their discontinuation. The main reason behind such unsuccessful clinical trials is the hormonal regulation and the compensatory mechanisms that resulted in an increase in IGF ligands and insulin levels as a feedback mechanism to enhance their actions, especially at times when the receptor is not blocked by an antibody [67, 68]. Yet, the clear connection between insulin and the IGF ligands is also a double-edged weapon that is useful in terms of blockade of both receptors whereas it is also responsible for dysregulation of blood glucose levels [67, 68].

Another Important Player in the Axis: IGFBPs

Insulin-like Growth Factor Binding Proteins (IGFBPs) Structure

The structures of all IGFBPs are similar with minute differences; they are composed of two significant N- and C-termini and a flexible linker domain [88]. Each terminus is stabilized by intramolecular disulfide bridges where six and three bonds are present in the N- and C-domains respectively, with the exceptions of IGFBP-6 which has five bonds in the N-terminus, and IGFBP4 which has an additional disulfide bond in the linking domain, this structural difference resulted in higher binding affinity of IGF2 to IGFBP-6 [88, 89]. NMR analysis of these structures is challenging yet what is currently known about these proteins is the necessity of the presence of both the N- and C-termini for proper functioning and maintenance of high binding affinities to the ligands [89, 90].

Physiological Role of IGFBPs

IGF concentration in serum is tightly regulated by six IGFBPs that have high affinities to IGF ligands [91]. Among the 6 IGFBPs, circulating IGFs have a superior affinity to IGFBP3 to a degree that IGFBP3 form predominates in human serum rather than free IGFs [91]. IGFBP5 also binds to the IGF ligands but to a lesser extent [92]. These complexes then bind to the acid-labile subunit (ALS) forming ternary complexes that act as storage pools for the IGF ligands regulating its concentration in plasma [90, 92]. Yet, these complexes fail to reach target tissues directly, unlike the remaining members of the IGFBP family (IGFBP2-4&6) which still show affinities for the ligands but to a much less extent [89]. The advantage of the latter binding proteins is their ability to form tiny complexes that can successfully be transported to tissues where the ligands are needed [89].

The IGFBPs contribution to the IGF pathway is huge and provides a reservoir for the ligands where this provides a mechanism of regulation for the circulating IGFs' concentrations, as well as, a method of increasing their half-life in circulation extending up to 19 h rather than their extremely short-lived nature of 10–12 min if found alone in circulation [89, 93]. Another incompletely understood function of these binding proteins includes the magnification of the actions of the entire IGF pathway, yet these molecules are also continuously showing several other significant effects unrelated to their usual IGF-related functions [89, 93]. The different functions induced by the IGFBPs in the body and the common assumption of their role in the tight regulation of the ligands further emphasize the complexity of the IGF pathway, making these regulatory binding proteins a questionable element in the pathway that needs further investigations [89, 93–95].

Pathological Role of IGFBPs: A Special Emphasis On BC

The most widely discussed binding protein is IGFBP3, which has been proven to play a vital role in regulating IGF1 levels, and accordingly tumorigenesis and tumor resistance to treatment if a downfall in the expression of the binding protein occurs [94, 96, 97]. Yet, recently another layer of

complexity has started to be unraveled concerning the role of IGFBP3 in the malignant transformation process. IGFBP3 was reported to have an intrinsic oncogenic activity that has been associated with C-peptide levels which are known for their direct association with a mortality rate among BC [98]. Consequently, an increased serum level of IGFBP3 has directly correlated with higher mortality rates and more aggressive BC phenotypes [98]. Mechanistically, it was revealed that binding protein has intrinsic modulatory effects on cellular growth and viability rather than its effect on IGF ligands and IGF signaling cascade. Yet, its interaction with other oncogenic proteins and signaling pathways has positively contributed to the role of IGFBP3 in BC [99]. For instance, the IGFBP3 role in reducing BC aggressiveness through the NF-κB pathway has also been documented [99]. Collectively, such contradictory actions of IGFBP3 have urged physicians to avoid considering IGFBP3 expression solely as a prognostic marker for BC patients. Yet, relating its expression level with other members of the IGF signaling pathway (e.g., IGF ligands) is essential to establish a reliable prognostic signature that could be related to BC progression. It was stated that a reduction in IGFBP3 accompanied by high plasma concentration of IGF1 is a combined feature characteristic for most BC patients [94].

Up-regulation of IGFBP3 or IGFBP5 has been shown to antagonize oncogenic IGF1 effects *in-vivo* and *in-vitro* where growth inhibitory effects and reduction in tumors' aggressiveness were demonstrated in MCF-7 cell lines and animal experiments [94].

IGFBP2 is also known to induce tumor-promoting effects of IGF1 and is associated with tamoxifen resistance and its potential immunomodulatory function, making it an important potential target in overcoming breast malignancy [100]. In case of IGFBP4, it was reported that IGFBP1 strictly inhibits IGF1 induced mitogenic activity [94]. Collectively, all studies report the association of IGFBPs to cancer progression through its tight regulation of IGF ligand. Yet, other modulatory effects of IGFBPs through their interaction with other signaling cascades are to be explored where IGFBPs could be in another position in the malignant transformation process without the association with any of the components of the IGF pathway [94, 100].

Clinical Significance of IGFBPs

Differential expression IGFBPs depends on the underlying BC subtype where IGFBP2 was reported to be more prevalent in ER^+ BC patients and cell lines whereas, IGFBP3 overexpression seems to be a feature of ER^- BC patients and cell lines [94]. While IGFBP4 elevated expression was evident in $ER^{-/+}$ BC subtypes. *In-vivo* studies showed similar results where overexpression of IGFBP3s was evident in the ER^+ subtype. Nonetheless, its association with poor prognosis and metastasis was additionally noted. On the other hand, IGFBP4 and IGFBP5 were the predominant forms in ER^- cells, and unlike IGFBP3, these binding protein levels increase with the increase in ER and/or PR [94]. Concerning the diagnostic potential of IGFBPs in BC, IGFBP2 is the one with high diagnostic value breast carcinogenesis. Attention should be drawn to the fact that the actions of this binding protein solely result in promoting anti-apoptotic effects, increase in mitotic activity, and indirect inhibition of regulatory IGF2R which is involved in reducing IGF2 induced cell growth [100].

IGFBPs As Promising Therapeutic Targets for Cancer Patients

Extensive research revolving around the role of IGFBPs in TNBC was performed. This is because IGFBPs have other signaling pathways they modulate other than the IGF axis. For instance, IGFBPs' modulatory role on other RTKs such as EGFR and its downstream target sphingosine 1-phosphate (S1P) is vital in stimulating cellular growth rates within the breast tissue [100]. Under physiological conditions this process contributes to normal growth yet in TNBC, providing malignant cells with another highly activated oncogenic signaling pathway notoriously leads to disease progression [100]. Accordingly, It was deduced that trio-inhibition of S1P, EGFR, and IGFBP3 in TNBC patients would have more potent anti-tumor effects than solely targeting each member separately; Further highlighting the urgent need to identify all other growth-promoting signaling cascades downstream the IGFBPs to be able to develop an appropriate therapeutic approach [100].

Recombinant IGFBP3 antibody was also provided as a possible therapeutic weapon to fight BC. Successfully, IGFBP3 antibodies resulted in a significant anti-growth activity of tumors alongside inhibition of the pro-mitogenic actions. Yet, dual-targeting IGFBP3 with another combined receptor inhibitor such as IGF1-R and/or HER2 did not provide the synergistic effects expected [101].

IGF1R is the Main Receptor for Such a Tenacious Signaling Pathway

Upon stratification of the IGF signaling pathways, it was shown that IGF1R is one of the main receptors regulating all the oncogenic signaling pathways drawn downstream the IGF/IGFBP interplay. For instance, it was previously reported by our research group and others that JAK/STAT, RAF/MEK/ERK, and PI3K/AKT/mTOR are dominant oncogenic signaling pathways drawn downstream IGF1R [102, 103]. In parallel, several new methods of inhibition were reported to trim IGF1R activity such as the incorporation of antisense oligonucleotides directed towards the IGF1R and/

or genetic interference interventions where genes encoding the receptor are silenced through specific RNA molecules [67, 104]. Although such approaches still require intensive research especially in developing appropriate delivery methods. Yet, these methods have been tested on different tumor types including BC [104].

Non-coding RNAs (ncRNAs): Potential Tool in the Battle of the IGF Axis

The initial discovery of lin-4 (microRNA) miRNA and innovative sequencing RNA technologies uncovered a critical class of regulators known as non-coding 'ncRNAs' [105, 106]. ncRNAs are encoded by the human genome and are further categorized into several types, long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). The role of ncRNAs in the regulation of the IGF signaling pathway is prevailing where several *in-vitro* studies presented the different effects these molecules induce on IGF1R expression where the ability of ncRNAs to modulate the expression of different IGF members in cancer increased the need to identify and analyze the functions of these molecules [107–109]. Sequencing analysis also revealed that dysregulation in the normal expression of ncRNAs is associated with altered mitogenic activity, angiogenesis, drug resistance, and metastasis via EMT induction which are the same complications resulting from IGF1R over-activation [107]. The variation in ncRNAs expression levels by different tumor types, as well as their relative stability and tissue-specificity significantly enhanced the molecules' diagnostic and prognostic potential [110, 111]. In such context, analyzing the different ncRNAs that are known for tuning members of the IGF axis and correlating their expression to the stage of BC is essential to determine valid conclusions regarding their clinical significance, not only as potential therapeutic targets but also as prognostic and diagnostic measures capable of monitoring the IGF axis. Hence, positively contributing to targeted therapy approaches since the overexpression of IGF1R by BC tumors seems to vary among individuals.

MicroRNAs (miRNAs):

miRNAs are currently a research hotspot, this is due to their normal regulatory roles along with, the aggressive effects and pathologies that arise from their dysregulation [112]. These molecules have the unique ability to promote or suppress gene expression via messenger RNA (mRNA) binding and thus can contribute to BC progression and development, however, some miRNAs may have both oncogenic and tumor-suppressive properties [113]. Consequently, examining the co-expression pattern of several miRNAs is more accurate than an individual expression of these molecules when it comes to their role as BC biomarkers [114] The serum stability of miRNAs and their variability in circulating tumor cells further unraveled the potential diagnostic and prognostic role of these molecules in different cancer types [111, 115]. Additionally, another study showed an elevated level of exosomes and exosomal miRNA in plasma of HER2 and TNBC patients, where Stevic and comrades found that miRNAs are also differentially expressed in exosomes, hence confirming the diagnostic potential of the molecules [116]. The role of miRNAs in metastasis has been extensively studied where several studies linked miRNA's dysregulation to the metastatic nature of BC [117]. This is suggested to occur through modulation of certain targets as E-cadherin, IGF1R, CD44, and p65 which activate various signaling pathways responsible for mediating cellular motility and invasiveness which include NF-κB and TGF-β signaling [118–121]. Other pathways regulated by miRNAs are continuously being discovered, therefore highlighting their therapeutic potential [112, 122].

MicroRNAs Regulating IGF Signaling Pathways

The discovery of miRNAs modulating this axis is critical due to the complexity of the IGF system. Correlating the expression of certain miRNAs to IGF1R expression (or other IGF members) as well as, their downstream effectors might provide useful information about the potential clinical role of these molecules. Mechanisms by which miRNA may contribute to cancer include mutations to miRNA binding sites which may potentiate tumorigenesis [123] Leukocyte recruitment and metastatic regulation further revealed an interlinkage between miRNAs and the IGF axis in BC where miR-126 negatively regulated IGFBP2 metastatic ability [124]. Furthermore, miR-630 expression is hindered by IGF1R upregulation which was observed in HER2 metastatic BC and TKI resistant BC, showing how this pathway further complicates the status of BC patients [125]. Interestingly, IGFBP6 is suggested to play a role in the regulation of miRNAs that promote EMT characteristics [126]. miRNAs can also be modulated by other genes where the suppression of tumor suppressor miRNAs was stimulated by mutations of p53, which amplified IGF1R expression and increased AKT activation [127]. The prognostic role of miRNAs is evolving where exosomal miRNAs expression may be characteristic for BC subtypes as HER2 and TNBC [116]. A detailed list of miRNAs regulating different members of the IGF pathway, as well as, the potential therapeutic/prognostic role of the different miRNAs has been summarized in Table 3 and Table 4. The tables also show the potential roles of these miRNAs in metastasis and resistance to therapy.

Long Non-coding RNAs (LncRNAs):

These molecules have been gaining quite popular because of their involvement in orchestrating numerous signaling pathways essential for normal development [128, 129]. There is a wide array of functions displayed by lncRNAs reported in literature where these molecules are known to modulate gene expression via three mechanisms 1) transcriptional regulation where lncRNAs act as decoys or scaffold molecules that stimulate/suppress gene expression [130, 131]. Furthermore, lncRNAs act as competing endogenous RNA molecules where they serve as sponges for miRNAs [132, 133]. 2) post-transcriptional regulation includes the ability of lncRNAs to regulate the translation and stability of mRNA, along with their ability to bind to proteins and induce their stability [134, 135]. 3) epigenetic regulation where lncRNAs can alter DNA methylation, plus, acetylation and ubiquitination of histones. lncRNAs can also bind to and remodel chromatin modification complexes and hence affect gene expression [136–138]. The stimulatory role of lncRNAs in BC progression and metastasis is inevitable and is an active area of research [139, 140]. This is due to the ability of the molecules to regulate several BC hallmarks such as cellular proliferation, metastasis, angiogenesis, colony-forming ability, and most significantly, EMT and chemoresistance [128, 141, 142]. The first oncogenic lncRNA associated with tumor initiation and migration was HOTAIR [138]. Whereas, the downregulation of tumor suppressor lncRNAs as NKILA are also linked to BC development and invasiveness [143]. The downstream metastatic effects induced by lncRNAs aberrant expression include the activation of STAT3 and nuclear factor-κB, besides TGF-β upregulation which is an important mediator of BC EMT [143–145]. The differential expression of lncRNAs in BC subtypes and their subsequent effects on oncogenic mediators suggest an important prognostic, diagnostic and therapeutic role of these molecules [146]. Recently, lncRNAs have entered the equation of ncRNAs regulating the IGF signaling pathway where several studies found a correlation between the expression of certain lncRNAs and the different mediators of the IGF pathway.

In two different animal models, lncRNA NR2F1-AS1 facilitated tumor growth and metastasis via sponging miRNA-338-3p, resulting in the upregulation of IGF1R and ERK hyperactivation [129]. Another interplay between members of lncRNAs and the IGF1R was recently discussed by Guo et al. where lncRNA TINCR acted as a sponge for miR-589-3p: a negative regulator of IGF1R [132]. Restoration of miR-589-3p partially abolished BC hallmarks initially induced by TINCR in vitro. This is suggested to occur via inhibition of the IGF1R/AKT pathway which is amplified by TINCR expression. [132, 163]. Finally, lncRNAs are being studied as biomarkers of disease pathology, prognostic indicators, and possible therapeutic targets [129, 164].. Deciphering their underlying mechanisms might be essential in inhibiting cancer development and metastasis. This is further analyzed in Table 5 which summarizes the various lncRNAs regulating different members of the IGF axis and their potential clinical roles.

Are NcRNAs the Candidates of Choice in Targeting IGF?

Since direct targeting of the receptor had its challenges, gene regulation by ncRNAs may represent an alternative approach when dealing with the complex pathway. The entanglement of the axis with other receptors in BC as previously discussed, as well as, with metastasis and EMT shows how targeting this axis via non-conventional ncRNAs might be useful in diminishing BC hallmarks in IGF1R overexpressed tumors. Figure 2 presents the different ncRNAs influencing the IGF axis. Although this may sound good in theory, the clinical application of ncRNAs is complex and gives rise to myriads of unanswered questions and possibilities. Naked miRNA application was found to be inefficient due to various reasons which include rapid degradation by nucleases, low uptake by tumor cells, and low tissue specificity (due to their bulkiness and negative charge) [165]. To overcome these challenges, suitable delivery vehicles for miRNAs are required.

Several therapeutic approaches are currently being investigated, aiming to find a suitable delivery method that 1) guarantees the specific delivery of ncRNAs to their target sites 2) protects them from degradation by nucleases 3) ensures high cellular uptake by the correct targets [166]. Recent in vitro studies emphasized the potential role of exosomes as nano delivery vectors for miRNAs where genetically modified mesenchymal stem cells were capable of secretion miRNA-containing exosomes [167–169]. These miRNAs loaded exosomes repressed cellular migration and invasion, in addition to reducing the expression of EMT-related proteins and genes [168]. Animal studies have also confirmed the potential of miRNA-loaded exosomes in reducing BC tumorigenicity [169, 170].

This may provide a new approach for miRNA delivery, yet still requires deep investigations. The usage of nanoparticles (NPs) as vectors for miRNA delivery has been emerging throughout the years, one of the main reasons behind such a phenomenon is that NPs can easily be modified, hence facilitating tumor targeting [177]. Different types of NPs can be used to encapsulate miRNAs, where nanoparticles made up of poly (lactic-co-glycolic acid) conjugated with polyethylene glycol can be loaded with anti-cancer drugs, as well as, miRNA inhibitors [178]. This accordingly results in a decrease in cellular proliferation in vitro and reduces tumor growth in mice. What's more is the ability of these

Table 3 Expression Signature, prognostic value and therapeutic value of miRNAs regulating different members of the IGF axis in breast cancer

miRNA	*Nature*	*Expression in BC*	*Correlation to IGF axis*	*Prognostic value (yes/no)*	*Association with metastasis*	*Therapeutic value*	*Resistance to Therapy*	*Reference*
microRNAs- 98, let-7c & let-7 g	Tumor suppressor	Downregulated	Downregulated by IGF1 induction	Not known	No known association	May be targeted	No known association	[147] [147]
microRNA-122	Tumor suppressor	Downregulated	Downregulates IGF1R	Important for prognosis	No known association	May be targeted	No known association	[148]
miR-424(322) & miR-503 (family: 16)	Tumor suppressor	Downregulated	Downregulates IGF1R	It Maybe of prognostic value	No known association	May be targeted	Low expression levels of this miRNA family promotes chemoresistance	[149]
microRNA 199a-5p	Tumor suppressor	Downregulated	Downregulates IGF1	No known prognostic value	positive	May be targeted	No known association	[150]
microRNA-375	Tumor suppressor	Downregulated	Downregulates IGF1R	No known prognostic value	No known association	May be targeted in trastuzumab-resistant HER2 positive cells	Downregulation of the miRNA is linked to trastuzumab resistance	[151]
microRNA-126	Tumor suppressor	Downregulated	Downregulates IGFBP2	No known prognostic value	Enhanced metastasis when the miRNA is downregulated	May be targeted in metastatic BC	No known association	[124]
microRNA-100	Tumor suppressor	Downregulated	Downregulates IGF2	No known prognostic value	May enhance metastasis through IGF2 induced actions	May be targeted in metastatic BC	No known association	[152]
microRNA-98-5p	Oncogene	Upregulated	Downregulates IGF1	No known prognostic value	No known association	May be targeted	No known association	[119]
microRNA-29a	Oncogene	Upregulated	miR-29a may be the downstream target for IGF-1R	Poor prognosis for hyperinsulinemia patients	induces metastasis through IGF1R/ERK axis	May be targeted	No known association	[153]
microRNA-148-3p:	Tumor suppressor	Downregulated in ER + BC	Downregulates IGF-1R Upon treatment with melatonin	Not known	Decreases angiogenesis upon melatonin transfection	May be targeted	No known association	[154]
microRNA-630	Tumor suppressor	Downregulated in HER2 resistant cells	IGF1R	Prognostic for HER2 resistance	Increased metastasis	May be targeted HER2 resistance	Low expression levels of miR-630 is linked to chemoresistance	[125]
miR-148a and miR-152	Tumor suppressor	Downregulated	Targeted by IGF1R	Prognostic for TNBC	Enhance angiogenesis	May be targeted in TNBC	No known association	[155]

Table 4 Expression Signature, prognostic value and therapeutic value of miRNAs regulating different members of the IGF axis in breast cancer

miRNA	*Nature*	*Expression in BC*	*Correlation to IGF axis*	*Prognostic value (yes/no)*	*Association with metastasis*	*Therapeutic value*	*Resistance to Therapy*	*Reference*
microRNA-99a	Tumor suppressor	Downregulated	Modulates IGF1R	Yes	Not known	May be targeted	No known association	[156]
microRNA-30a	Tumor suppressor	Downregulated	Downregulates IGF1R	Not known	Not known	May be of therapeutic importance for patients with p53 R273H	No known association	[127]
miR-203a-3p	Overexpressed	Upregulated	Modulates IGF1	Not known	Not known	Not known	No known association	[157]
Let-7a-3	Tumor suppressor	Downregulated	Downregulates IGF2 mRNA binding protein and IGF2	Not known	Not known	Not known	No known association	[158]
microRNA-7	Tumor suppressor	Downregulated	Downregulates IGF1R	Yes	Promotes metastasis	Potential therapeutic targeting in aggressive TNBC	No known association	[159]
miR-152-3p	Tumor suppressor	Downregulated	Downregulates IGF1R via melatonin transfection	Yes	Promotes angiogenesis	Targeting this axis via melatonin showed promising results	No known association	[160]
microRNA-503	Tumor suppressor	Downregulated	Downregulates IGF1R	Not known	Needs further studies to validate its inhibitory effect on metastasis	May be targeted	No known association	[149]
miR-424(322)/503	Tumor suppressor	Downregulated	Downregulates the expression of IGF1R	Not known	Not known	Potential therapeutic target in BC resistant to chemotherapy	Low expression levels of this miR family is linked to chemoresistance	[161]
microRNA-320a	Tumor suppressor	Downregulated	Downregulates IGF1R	Not known	Prevents metastasis	Not known	No known association	[162]

miRNA-loaded nanoparticles to restore cellular response to therapy in TNBC [178, 179]. Similarly, NP encapsulating small interfering RNA directed towards lncRNAs regressed BC hallmarks in both cell lines and mice [180, 181]. Combination therapy via NPs has also been achieved where doxorubicin and tumor suppressor miR-34a were co-encapsulated into hyaluronic acid and chitosan NPs, hindering migration and stimulating anti-tumor effects both in vivo and in vitro [182]. Lipid NPs conjugated with hyaluronic acid and loaded with miRNAs have also reduced P13/AKT and MAPK signaling, hence suppressing cellular proliferation and migration in HER2 metastatic BC [183]. Interestingly, ultrasound-induced nanobubble cavitation enhances the specific delivery of NPs loaded with miRNAs/lncRNA inhibitors to target tumor tissues which might be useful for TNBC [184, 185]. Moreover, lncRNAs incorporation in NPs has resulted in sponging oncogenic miRNAs and hence anti-tumor effects and immune stimulation [186]. Magnetic nanoparticles have also been used to deliver lncRNA inhibitors in human gliomas where lncRNA HOTAIR was significantly under-expressed, reducing tumor migratory ability and also limiting its oncogenic downstream effects [187]. On top of that, liposomal spherical nucleic acids have demonstrated great potential as vectors for lncRNA delivery [188]. Viral

Table 5 Expression Signature, prognostic value and therapeutic value of lncRNAs regulating different members of the IGF axis in breast cancer

LncRNA	*Expression in breast cancer*	*Correlation to IGF access*	*Prognostic value (yes/no)*	*Association with metastasis*	*Therapeutic value*	*Chemoresistance*	*Reference*
Airn	Tumor suppressor	Downregulates IGF2R	Not known	Not known	Not known	No known association	[171]
FGF13-AS1	Tumor suppressor	Downregulates IGF2	Yes	Negative	May be targeted	No known association	[172]
N2RF-AS1	Oncogene	Induces IGF1	Not known	Promotes angiogenesis through downregulating miRNA-338-3p	May be targeted	No known association	[129]
IRAIN	Tumor suppressor	Downregulates IGF1R	Not known	Directly linked to BC metastasis	May be targeted	No known association	[173]
LINP1	Oncogene I	Induces IGFBP3	Not known	Not known	May be targeted	Promotes resistance to chemotherapy	[174]
SNHG7	Oncogene Overexpressed in BC	Downregulates by IGF1	Yes	Not known	Needs further studies	No known association	[175]
H19	Overexpressed	Induces IGF2	Not known	Not known	Needs further studies	No known association	[176]

vectors can also be utilized as ncRNA delivery vectors and have proven to be efficient in hepatocellular carcinoma, yet issues regarding their high immunogenicity, as well as, their ability to overexpress ncRNAs have to be resolved before their clinical usage [189, 190]. Therapeutic targeting of ncRNAs is clinically challenging and no valid conclusions about the use of ncRNA loaded-NPs can be reached due to lack of research and clinical trials. The roles of ncRNAs as biomarkers also still require further investigations and standardizations. Regarding the potential of these delivery methods in targeting the IGF system, a recent study succeeded in using modified lipid NPS as a vector for delivering siRNA against

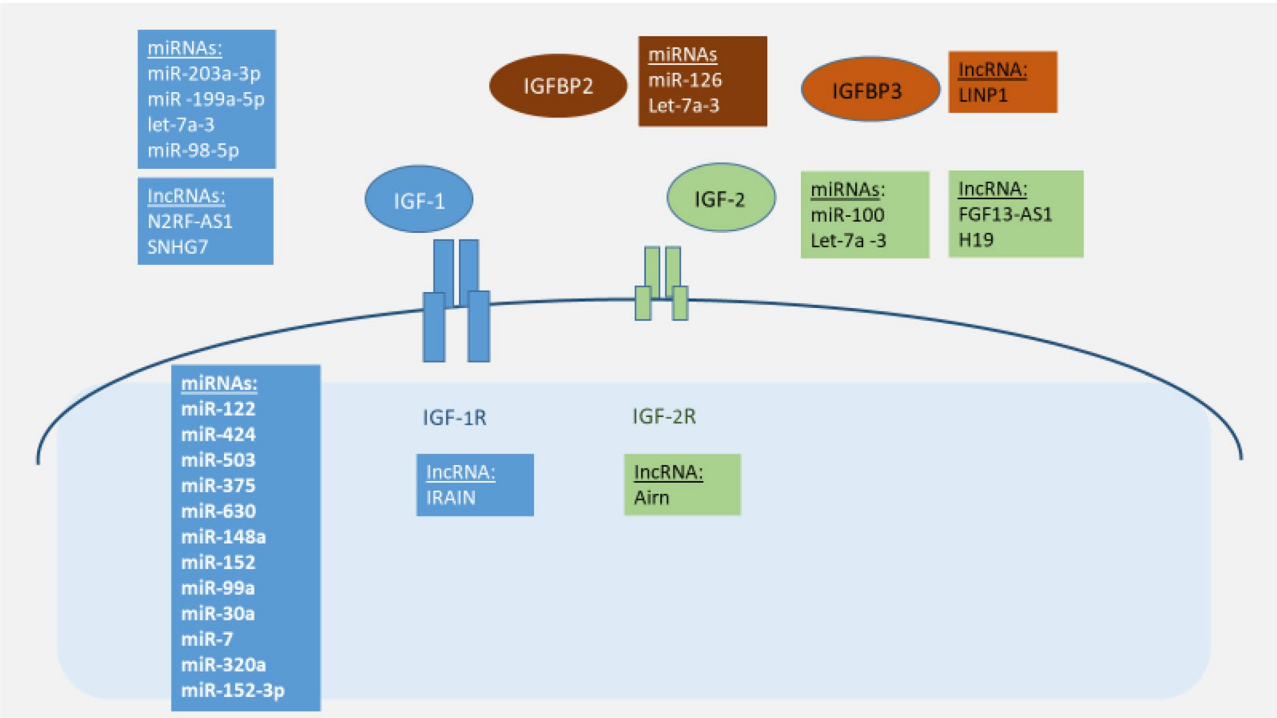

Fig. 2 A snapshot of important short and long non-coding RNAs regulating vital players of the IGF pathway. This figure represents representatives of non-coding RNAs (microRNAs and long non-coding RNAs) regulating IGF1, IGF2 ligands, IGF1R, IGF2R, IGFBPs

IGF1R, additionally, co-delivery of siRNA directed towards IGF1R along with chemotherapy via NPs repressed IGF1R expression, cellular viability and metastasis [191]

Future Recommendations

After decades of research, it could be confidently said that in oncology the Multiple-Targeting Approach is the winner in such battle. Therefore, research should be directed towards modulators that could multiply hit several members of the IGF pathway simultaneously. The best candidates theoretically on paper for such task are the ncRNAs. In addition, a novel class of ncRNAs has also been discovered and is gaining a lot of popularity, which is circular RNAs. Circular RNAs mediation of BC signaling pathways has been recently discussed yet the mechanisms by which they exert their actions are yet to be identified. For instance, circRAD18 has been recently linked to BC progression, specifically TNBC where it enhances the release of the IGF1 through hindering the effect of tumor suppressor miRNAs; hence, these molecules may represent potential therapeutic targets and may even play a bigger role in the regulation of miRNAs [133]. Additionally, the emerging role of phytochemicals in altering the expression of miRNAs and lncRNAs should also be taken into account when targeting the latter, for instance, hesperetin is a natural product isolated from citrus fruits and has shown promising anti-mitogenic activity *in-vitro* via controlling the expression of several lncRNAs as H19 [192]. Baicalein and Calycosin are another two plants that have also demonstrated similar effects on BC progression in vitro. Moreover, the use of nanoparticles and exosomes in targeting ncRNAs would provide numerous advantages yet still requires in-depth research [170, 179].

Conclusion

In this review, the authors shed the light on the need for standardized biomedical studies to evaluate the eligibility of ncRNAs as potential trimmers of the IGF signaling pathway that has been highly associated with metastasis, resistance, and poor prognosis among BC patients. Attempts to decipher this pathway have been going on for decades, yet it's clear that targeting single members of the IGF directly is not the solution, finding master regulators such as ncRNAs or phosphatases that tune the IGF axis and its numerous feedback mechanisms would be more propitious. This requires closely analyzing the pathological functions and signaling of the members of the pathway in BC and monitoring the questionable parameters. While countless ncRNAs are continuously being discovered, their exact mechanisms of action and affected downstream and upstream parameters need to be further elucidated in-depth to correctly identify new prognostic, diagnostic, and therapeutic targets. Suitable delivery methods for ncRNAs are also still lacking and require further research.

Moreover, this review highlights the resistance of the IGF pathway for possible inhibition and this spots the light onto the urgent need to extensively analyze all other factors that could be involved in the tumorigenic equation such as the TME cross talks with other signaling pathways and different mechanisms of resistance [193]. It is also clear how combination therapy is most likely the most suitable approach to target such a huge pathway and its downstream signaling pathways, also the insulin endocrinal resistance resulting from dysregulation of the receptor or the bioavailability of the ligand should be deeply analyzed to identify potential therapeutic targets. Nonetheless, additional research is required to develop different methods of inhibiting the pathway due to the disappointing results of clinical trials including monoclonal antibodies and tyrosine kinase inhibitors. While ncRNAs have been gaining more attention due to their interplay with several oncogenic mediators which include different members of the IGF system, clinical application of these molecules still requires further analysis to reach valid conclusions regarding their actual clinical significance. Lastly, due to the different roles, each member has in different subtypes of BC, personalized treatment code has become an urgent matter in oncology, specifically among BC patients.

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