

MiRNA and target oncogenes in Triple negative breast cancer; major players in age and ethnic related neoplastic events

Abstract

Approximately 15-20% of all breast cancers are referred to as triple-negative breast cancers (TNBC) which is a type of aggressive breast cancer lacking the immunohistochemical expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor-2, HER-2 with the direst prognosis; higher relapse risk and worse overall survival. An increased proportion of TNBC patients have higher rates of CNS and visceral metastases; particularly the lungs and brain, and to a lesser degree in bones. TNBC is more prevalent among younger women, particularly African, African-American, Latino and obese women. Inherited variation in the *let-7* binding site in the *KRAS* oncogene has been revealed to confer increased susceptibility to TNBC particularly in premenopausal, African-American and Hispanic women. About 15-40% of TNBC tumors have *BRCA*-related epigenetic down-regulation and increased expression of the inhibitors of *BRCA1* function. MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression post-transcriptionally by pairing to 3' untranslated regions (UTRs), coding sequences or 5' UTRs of target messenger RNAs (mRNAs), which in most cases leads to translation inhibition or mRNA degradation. These miRNAs function by engaging some target (especially HDACi, ZEB1, ZEB2, *EphA2*, etc) which in turn regulate epithelial-to-mesenchymal transition (EMT) associated with metastatic and aggressive disease, of TNBC. The expression of miR-21, miR-210, and miR-221 have been observed to be higher in the triple-negative (TN) subtype of breast cancer compare with corresponding healthy tissues; while the expression of miR-10b, miR-145, miR-205, miR-122a, miR-200a/miR-200b, miR-146b and miR-148a has been observed to be lower. Eight miRNAs are differentially expressed in metastatic tissues (miR-200b, miR-148a, miR-424, miR-125a-5P, miR-627, miR-579, let-7g, miR-101), with miR-424 and miR-125a differentially expressed only in the metastasis vs. primary tumor and in normal tissue vs. metastasis. Insights into the risk factors, differential miRNA and their target expressions may provide preventive measures, diagnostic tools and treatment options for TNBC.

Introduction

More than 1 million women worldwide are diagnosed with breast cancer each year of which 170,000 cases are defined as triple negative breast cancer [1]. Breast cancer afflicts approximately 1 in 8 women and is a leading cause of cancer-related mortality. According to Reis-Filho and Tutt [2], expression profiles of breast cancer exhibit a systematic variation and allow for the classification of breast cancer into five main groups: two estrogen receptor (ER) positive (luminal A and B) and three ER negative groups (normal breastlike, HER2 positive, and 'basallike'). Approximately 15-20% of all breast cancers are referred to as triple-negative breast cancers (TNBC) which is a type of aggressive breast cancer with the direst prognosis lacking the immunohistochemical expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor-2, HER-2 [3]. TNBC is more prevalent among younger women [4], particularly young African, African-American [2,5] and Latino women [2,6]. The prevalence of TNBC is also higher among young obese women [6-8]. A study carried out by Heneghan [9] revealed that an inherited variation in the *let-7* binding site in the *KRAS* oncogene conferred increased susceptibility to breast cancer particularly the 'triple negative' subtype. The *KRAS*-variant is found in almost 30% of premenopausal ER/PR/HER2 negative breast cancer patients, and in over 20% of unselected TN patients. According to this study, the *KRAS*-variant was also observed to be prevalent in African-American and Hispanic women. The term triple negative breast cancer encompasses several subgroups of breast cancer patients, including basal-like breast cancer (BLBC) [10] and rare tumors such as metaplastic tumors and adenoid-cystic tumors [11] of the breast whose biology is somewhat different from that of the high-grade invasive ductal TNBC. MicroRNAs (miRNAs) are involved in the regulation of

key cellular process including development, differentiation, cell proliferation, apoptosis, fat metabolism, angiogenesis, and inflammation [12]. MicroRNAs are a class of endogenous, small, non-coding RNAs that control gene expression by interacting with target mRNAs, resulting in either mRNA degradation or translational repression [13]. There is an increasing body of evidence showing that microRNAs (miRNAs) play pivotal roles in stem cell biology, differentiation and oncogenesis [2,14,15]. Tumor suppressor or oncogenic miRNAs have been described, and dysregulation in their expressions causes tumorigenesis [16,17], metastasis and a poor prognosis [18]. Altered miRNAs exert their effects by regulating an array of specific targets (table 1) which in turn determines migration and invasion as well as tumourigenicity, metastases and poor prognosis. The review article gives an overview on the major roles of miRNA and their validated targets in the emergence, metastases, prognosis and treatment of TNBC.

Characteristic features of TNBC

TNBC are characterized by a number of clinical and histological patterns [19,20,2,5,6]. Lehmann et al. [21] profiled more than 500 TNBCs classifying them in six different subtypes, according to intrinsic gene signature: basal 1 and 2, mesenchymal and mesenchymal stem cell-like, immunomodulatory and androgen pathway enriched. The characteristics features include the following: i) Because of the lack of estrogen receptor (ER) and progesterone receptors (PR) and of the human epidermal growth factor receptor 2 (HER-2), TNBC patients are insensitive to most currently available hormonal or ER-targeted and HER-2-based therapies. However, TNBC patients have a better response to chemotherapy in the neoadjuvant setting, in comparison to non-TNBC patients [22,23]. TNBC patients that are initially non-responsive have higher relapse risk and worse overall survival at 68% than responders who exhibit pathologic complete response at 94% overall survival [24]. They are managed only with standard chemotherapy treatment, which leave them associated with a high rate of local and systemic relapse[4]; ii) TNBC patients share several features with breast cancer susceptibility gene outcome-associated breast cancer [25-28]. According to Sorlie et al. [29], 15-40% of TNBC tumors have *BRCA*-related epigenetic down-regulation and increased expression of the inhibitors of *BRCA1* function. This indicates that the label “triple-negative breast cancer” describes a more heterogeneous subtype than other breast cancer subtypes [30,31]. More than 80% of *BRCA*-1 mutation carriers are triple negative and approximately 20% of breast cancer patients with mutations in *BRCA*-1 and *BRCA*-2 have deficiency in DNA repair, because normal *BRCA1* and *BRCA2* have an important role in DNA repair. TNBC patients who have problems with DNA repair make the mutation carriers more sensitive to DNA damaging agents; iii) TNBC are poorly differentiated, highly malignant, more aggressive, and with a poor outcome [32,19]. Women with TNBC are twice as likely as other women to develop distant metastases. An increased proportion of TNBC patients have higher rates of CNS, visceral; particularly the lungs and brain [33], and to a lesser degree in bones [22]and overall poor outcome despite therapeutic treatment [23]. This metastatic phenotype of TNBC is due in part to the high nuclear grade of TNBC tumors and results in shorter time to disease recurrence and shorter median survival time in of advanced metastatic TNBC [22,23]. Moreover, TNBC patients have the highest percentage of early local relapse, especially between the first and third year after diagnosis [34]. Because of these, TNBC patients have shorter survival; and iv) Altered expression of a number of proteins, oncogenes, tumor suppressor proteins and abnormal signaling pathways has been detected in TNBC. Key mediators of immunological response, proliferation, and neuronal signaling, such as *MYC*, *LYN*, *EGFR*, and *CEBPB*, are either amplified or overexpressed in TNBC in comparison to other breast cancer subtypes [35]. Epidermal growth factor receptor (*EGFR*) has been identified as a frequently overexpressed marker in triple-negative breast cancer [36].

Bio-genesis of microRNAs

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression post-transcriptionally by pairing to 3' untranslated regions (UTRs), coding sequences or 5' UTRs of target messenger RNAs (mRNAs), which in most cases leads to translation inhibition or mRNA degradation[37,38]. The mature miRNA molecules are produced in a multi-step process. The DNA sequence is transcribed by RNA polymerase II into a single stranded RNAmolecule by hairpin structures known as primary transcripts or pri-miRNAs. Thepri-miRNAs are processed (cutted) into the nucleus by RNase III Drosha into 70100nucleotides long fragments called pre-miRNAs. The pre-miRNA molecule is then actively transported to the cytoplasm by a carrier protein. Here, an additional step mediated by the Dicer, generates a double strand. RNA (dsRNA) approximately 22 nucleotides long, including the mature

miRNA guide (3p arm) and the complementary passenger strand (5p arm). Once completed the processing steps, through this mechanism that is not fully characterized yet, the mature miRNA is able to regulate gene expression at post-transcriptional level, binding through partial complementarity the 3' untranslated region (3' UTR) of the target mRNA, and mainly leading to either mRNA degradation or translation inhibition [39]. Depending on the targeted mRNAs, the miRNA action ultimately results in reduced protein levels and profound consequences on cellular homeostasis. In 2004, an *in silico* study showed that more than half of miRNAs map to genomic regions that are frequently altered in cancer [40]. This includes: loss of heterozygosity regions (LOH) (e.g. miR-15a/16-1), amplified regions (e.g. miR-17-92 cluster, miR-155) and breakpoint regions and fragile sites (FRA) (e.g. let-7 family members)

MicroRNA and Epithelial-to-Mesenchymal Transition (EMT); players in TNBC and metastases

Tumorigenesis and progression can be seen as evolutionary processes, in which the transformation of a normal cell into a tumor cell involves a number of limiting genetic and epigenetic events that occur in a series of discrete stages [41]. Both functional and observational data implicate alterations in histone modifications, DNA promoter methylation, and non-coding RNA expression in carcinogenesis [42]. On the other hand, due to the wide range of biological functions of miRNAs, analyzing changes in overall miRNA expression levels within human tumors has helped identify miRNA signatures associated with diagnosis, staging, progression, prognosis, and response to treatment [43]. Epigenetic, defined as heritable change in gene activity that is independent of DNA sequence, play a prominent role in the initiation and progression of cancer. The main epigenetic events that regulate tumor-associated genes are linked to aberrant hypermethylation of tumor-suppressor genes, global DNA hypomethylation and post-translational modifications of histones and other proteins by acetylation and/or phosphorylation. MiRNAs can be target of epigenetic events that, in some instances, can explain the perturbation of miRNA expression in cancer [44].

EMT (epithelial to mesenchymal transition) is the process by which epithelial cells convert to mesenchymal cells and is essential in embryonic development. It appears that aberrant activation of EMT later in life drives cancer progression, and is involved in highly aggressive, poorly differentiated breast cancers with increased potential for metastasis and recurrence [45]. The miR-200 family appears to be one of the most interesting players in TNBC biology; it was previously described as up-modulated in breast cancer where its over-expression was correlated with lymph node positivity and metastasis [46]. Among the different breast cancer subtypes, the miR-200 family was shown to be down-modulated in metaplastic carcinoma [47], one of the most mesenchymal and undifferentiated breast cancers characterized by TNBC phenotype. Furthermore, the miR-200 family, in particular miR-200c, inhibits cancer cell migration [48], invasion [49] and reverts the anoikis resistance [50,51] that is frequently observed in aggressive carcinoma cells where it correlates with EMT [52,53]. More so, low expression levels of miR-200c associated with poor response to chemotherapy [54] and radiotherapy [55]. miR-200a and miR-200b have been shown to play a number of critical functions in mammary stem cells [56-58]. They have been shown to inhibit EMT, which is important during development, progression and metastasis [59]. A low expression of *E-cadherin* is indicative of EMT. The miR-200a/miR-200b have been shown to increase *E-cadherin* expression through downregulation of its transcriptional repressors *Zeb1*, *Zeb2* and the polycomb complex *Suz12* [58,60,61]. A study carried out by Collin-Burrow [62] demonstrated that HDAC inhibitor (HDACi) regulate microRNAs which in turn regulate the epithelial-to-mesenchymal transition (EMT) in triple-negative breast cancer (TNBC). This study assessed the microRNA expression profiles of two TNBC cell lines following treatment with HDACi identifying a number of up- and down-regulated microRNAs. The overall microRNA profile after HDACi treatment was indicative of a less aggressive phenotype. In this study, miR-126, miR-146b-5p, miR-192, miR-194, miR-215, miR-342.3p and miR-424 were observed to be increased while miR-100, miR-106b, miR-125b, miR-331-3p, Let-7i were decreased. Treatment with HDACi also resulted in increased expression of *E-cadherin* as well as decreased migration of TNBC indicating a reversal of the EMT phenotype (which is associated with metastatic and aggressive disease, of TNBC).

Recent data described miR-181a, which is overexpressed in TNBC [63], as metastatic miR. Down-modulation of miR-181a inhibits TGF- β mediated EMT, invasion and migration, also reverting anoikis resistance in breast cancer cells; high levels were then associated with shorter disease-free survival of breast cancer patients, who tested negative for ErbB2 amplification [63]. Moreover miR-181a/b expression sensitizes to cisplatin [64] and to PARP inhibitors [65] by dampening the DNA double-strand-

breaks repair, thus representing a possible marker of BRCAness. Quantification of miR-181a levels could then be useful for determining basal-like and TNBC patients' sensitivity to platinum-derived compounds and who could really benefit from PARP inhibitors treatments.

Some miRNAs including miR-141-3p, miR-203a, miR-548c-3p, miR-607 and miR-96-5p were significantly overexpressed, whereas miR-181a was significantly underexpressed in the triple-negative primary breast cancers versus double positive cases. Furthermore, according to the reports of Radojicic et al. [66], the expression of miR-21, miR-210, and miR-221 have been observed to be higher in the triple-negative (TN) subtype of breast cancer compare with corresponding healthy tissues; while the expression of miR-10b, miR-145, miR-205, miR-122a, miR-200a/miR-200b, miR-146b and miR-148a has been observed to be lower. Let-7c, miR-27b, miR-22, miR-143, miR-30b and miR-30d showed significantly decreased expression levels in all breast cancer cell lines (ER α -positive MCF-7 and T47D and triple-negative MDA-MB-231) compared with non-tumorigenic epithelial MCF-10A cells [4]. TNBC-associated lymph node metastasis and normal tissues analyses lead to the identification of a set of six miRNAs which were differentially expressed in the metastatic tissues (miR-424, miR-125a-5P, miR-627, miR-579, let-7g, miR-101), with miR-424 and miR-125a differentially expressed only in the metastasis vs. primary tumor and in normal tissue vs. metastasis, respectively [67]. Interestingly, two miRNAs (miR-200b and miR-148a) have been predicted in targeting the E2F pathway that is active in poor-prognosis and metastatic breast cancer [4]. miR-203 inhibits cell proliferation and migration, while miR-141-3p inhibits metastasis; overexpression of miR-548c-3p is associated with poor prognosis. miR-181a has been downregulated in other solid tumours, being involved in cellular transformation [1]. Up-modulation of miR-155 induces angiogenesis and tumor growth in breast tumours and metastasis, late-stage/high grade tumor and poor prognosis [68]. Interestingly, a significant proportion of TNBC show high expression of miR-155 (moreover, it was shown to be epigenetically regulated by BRCA1 and up-regulated in BRCA1-deficient or BRCA-mutant breast cancer [69] and stimulated by pro-oncogenic stimuli such as hypoxia, both risk factors for TNBC).

One of the most studied microRNA which consistently is found up-regulated in wide variety of cancers, including breast cancer, is miR-21 [70-72]. Up-regulated expression of miR-21 in breast cancer tissues is associated with advanced clinical stage, lymph node positivity, and low survival rate [70,72]. Some of the microRNAs have shown different expressions not only within the specific subtype of breast cancer but between distinct subtypes of breast cancer as well. MiR-21 is an oncogenic microRNA with anti-apoptotic potential which is directly involved in the growth, proliferation, and invasion of the tumor cells by inhibiting the activity of the tumor suppressor genes *PDCD4* (programmed cell death-4) and tumor suppressor tropomyosin-1 (TPM1) [73,74]. Expression levels of miR-21 and miR-29a were significantly higher in triple-negative tumor tissues than in luminal-B tumor tissues [75]. Another microRNA which has been reported as being expressed differentially in the TN tumors is miR-31. The down-modulation in TNBC cell lines, with particular attention to basal subtype, was partially attributed to epigenetical hypermethylation of LOC554202, the miR-31 host gene [66,76]. In addition to its role in the metastatic process, miR-31 overexpression also induces apoptosis and increases chemo- and radiosensitivity in TNBC cell lines by direct inhibition of PRKCE, a positive regulator of Bcl-2 [77]. Inverse correlation between miR-31 and Bcl-2 was found by exploiting a public dataset of breast cancer patients [78].

Table 1: miRNA expressions in Triple Negative Breast Cancer (TNBC) with relative validated targets and biological functions [79-81].

miRNA	target	Description/Biological function of miRs
miR-9	CDH1	miR-9, identified as a new "metastomiR" and activated by MYC and MYCN, directly targets CDH1, the E-cadherin encoding messenger RNA, leading to increased cell motility and invasiveness, activation of β -catenin signaling and upregulation of VEGF.
miR-10a/10b	HOXD10	TWIST transcription factor induces expression of a specific microRNA that suppresses its direct target and in turn activates another pro-metastatic gene, leading to tumor cell invasion and metastasis
	TIAM1	A mechanism for the regulation of Tiam1-mediated Rac activation in breast cancer cells
miR-19	TF	It target tissue factor (TF), a known promoter of cancer cell survival,

		angiogenesis, and metastasis. Therefore, miR19 may also play a tumorsuppressive role in breast cancer.
miR-21	TPM1	Suppression of miR-21 can inhibit tumor growth
	PDCD4	The tumor suppressor protein programmed cell death 4 (PDCD4) is regulated by miR-21, and it has been demonstrated that PDCD4 is a functionally important target for miR-21 in breast cancer cells
miR-31	ITGA5, RDX, RhoA, FZD3, M-RIP, MMP16, SATB2	miR-31 uses multiple mechanisms to oppose metastasis
	WAVE3	Reduction of metastatic potential
	PRKCE	Induction of apoptosis and enhancement of chemo- and radiosensitivity
miR-34a	AXL	Impairment of migration
miR-107/103	DICER	Dicer inhibition drifts epithelial cancer toward a less-differentiated, mesenchymal fate to foster metastasis
Let-7 family	PLC1	Tumor-suppressor function by negatively regulating EGF-driven cell invasion, viability, and cell cycle progression in breast cancer
	IL6	Inflammation activates a positive feedback loop that maintains the epigenetic transformed state
miR-146 and miR-146b-5p	BRCA1	control of BRCA1-mediated proliferation and homologous recombination
miR-155	SOCS1	miR-155 is an oncomiR in breast cancer, and it has been suggested that miR-155 may serve as a bridge between inflammation and cancer
	WEE1	miR-155 enhances mutation rates by decreasing the efficiency of DNA safeguard mechanisms by targeting of cell cycle regulators such as WEE1
	FOXO3a	Molecular links between miR-155 and FOXO3a affect cell survival and response to chemotherapy in breast cancer
miR-181a/b	Bim	Inhibition of anoikis
	ATM	Impairment of DNA double-strand-breaks repair
miR-182	PFN1	Inhibition of cell proliferation and invasion Induction of apoptosis
miR-199a-3p	Caveolin-2	Regulates cell proliferation and survival.Caveolin-2 is a member of a family of scaffolding proteins that coats plasma membrane invaginations(caveolae).
miR-200 family (a/b/c)	ZEB1, ZEB2	miR-200 stimulates differentiation in undifferentiated mammary epithelial cell line and inhibits EMT. Down-regulation of the miR-200 family may be an important step in tumor progression
	PLC1	Tumor-suppressor function by negatively regulating EGF-driven cell invasion, viability, and cell cycle progression in breast cancer
	SUZ12	The miR-200b-Suz12-cadherin pathway is important for cancer stem cell growth and invasive ability
	FN1, LEPR, NTRK2, ARHGAP19	miR-200c actively represses a program of mesenchymal and neuronal genes involved in cell motility and anoikis resistance
	EphA2	Inhibit epithelial-mesenchymal transition (EMT)-characteristic morphological changes in undifferentiated, non-tumorigenic mammary cells.
miR-203	BIRC5	Reduction of proliferation

	LASP1	Inhibition of migration
miR-205	E2F1; LAMC1	Reduction of proliferation, cell cycle and tumor growth
miR-221/222	FOXO3a	The miR-221/222 cluster targets FOXO3A to suppress p27kip1 also at a transcriptional level
	ESR1	Modulation of ER is associated with antiestrogen therapy
	TRSP1	miR-221/222 promote EMT and contribute to the more aggressive clinical behavior of basal-like breast cancers
	DICER	Dicer is low in ER-negative breast cancers, since such cells express high miR-221/222
miR-224	PAK4 and MMP-9	It has been associated with cancer progression and enhanced cell migration and invasion by increasing the expression of the proinvasive PAK4 and MMP9.
miR5425p		Associated with maintenance of the mesenchymal phenotype, a key characteristic of the TNBC phenotype and driver of cell motility and invasiveness.
miR-17/92	HBP1	The miR-17/92 cluster plays an important role in breast cancer cell invasion and migration by suppressing HBP1 and subsequently activating Wnt/-catenin

Prognostic and Therapeutic markers in TBNC

Although miRs that have been demonstrated to be implicated in the metastatic process might represent a possible therapeutic tool, there have been so far few reported successes in the development of miRNAs for use in therapy. There are two main strategies to target miRNAs expression in cancer. Direct strategies involve the use of oligonucleotides or virus based constructs to either block the expression of an oncogenic miRNA or to reintroduce a tumor suppressor miRNA lost in cancer [82]. The indirect strategy involves the use of drugs to modulate miRNAs expression by targeting their transcription and their processing. Distinct changes in miRNA expression profiles and proteomes have been observed following glyceollins treatment of the triplenegative breast carcinoma cell line, MDAMB231. Glyceollins, previously described as antiestrogenic agents, also exert antitumor activity in triplenegative breast carcinoma cell systems [81]. Decreased expression of miR5425p, as well as an increased expression of NME1, indicates a reversal of EMT and a suppression of metastasis by glyceollins. A second target identified via proteomics approach is vimentin, whose expression was downregulated more than 13fold by glyceollins. Vimentin is a marker for epithelial to mesenchymal transition and is highly expressed in numerous TNBCs and cell lines including MDAMB231 [83]. Vimentin is a proven target of miR30d [84] and a predicted target of miR138, both found to be significantly increased by glyceollins. These data suggest that the effects of glyceollins on TNBC cell lines are achieved via regulation of miRs, which in turn regulate known oncogenes and tumor suppressors [81]. Among the most downregulated miRs following treatment with glyceollins were 193a5p, 197, 224, 4865p, and 5425p, all of which have been associated with cancer progression [81].

Further analysis of miR-342 and miR-520g in 95 breast tumors revealed that miRNA-342 expression was highest in ER α and HER2 positive Luminal-B tumors and lowest in TNBC tumors whereas miRNA-520g expression was elevated in ER α - and PR-negative tumors. The association of specific miRNAs with ER, PR and HER2 status indicates a role for these miRNAs in disease classification of breast cancer. Decreased expression of miRNA-342 in TNBC tumors, increased miRNA-342 expression in the Luminal-B tumors, and downregulated miRNA-520g in ER- and PR-positive tumors indicate that deregulated miRNA expression is not only a marker for poorer prognosis of breast cancer, but it could also present an attractive target for therapeutic intervention [85]. Recent studies [86-92] have shown that a number of miRNAs are involved in regulation of ER α expression and ER α -mediated signaling in breast cancer cells.

A number of known microRNAs have been identified as prognostic in breast cancer, including the Triple Negative subgroup, where a set of four microRNAs were found to associate with overall survival (OS). In detail, up-regulation of miR-16, miR-155 or miR-374 correlated with better prognosis while down-modulation of miR-125b correlated with worse prognosis. Furthermore, a set of seven microRNAs were associated with distant disease free survival (DDFS). Three "risk-associated" miRs (miR-125b, miR-655

and miR-421) and four “protective” miRNAs have been identified (miR-16, miR-374a, miR-374b and miR-497) which negatively and positively correlated with DDFS, respectively [93]. However, these signatures require further validation in a second set of TNBC samples. MiR-210, over-expressed in TNBC compared to ER+ [94], was described as an independent prognostic factor in TNBC; low levels of miR-210 showed better distant-free-survival (DFS) in TNBC and no relapse in the following 5 years after surgery in node negative TNBC [95]. MiR-34b, a member of the p53-regulated miR-34 family [96], is negatively associated with DFS and OS [97]. However this observation is not consistent with previously reported tumor-suppressor roles of the miR-34 family in cancer. Interestingly, there is a positive correlation of miR-25, miR-10b, miR-130b, miR-1274a, and miR-Plus-1030 and a negative correlation of miR-29c, which is downregulated in ER/cytokeratin 5 and 6 positive tumors, with proliferation in a set of lymph node negative breast cancer [98], since proliferation represents the strongest single prognostic indicator in this group of patients [99].

XBP1 has been reported to be activated in TNBC and has a pivotal role in the tumorigenicity and progression of this human breast cancer subtype with identified XBP1's control of the HIF1 α transcriptional program as the major mechanism. In breast cancer cell line models, depletion of XBP1 inhibited tumour growth and tumour relapse and reduced the CD44^{high}CD24^{low} population. Tumour cells expressing CD44^{high}CD24^{low} have been shown to mediate tumour relapse in some instances [100–102]. XBP1 pathway activation correlates with poor patient survival in TNBC patients, indicating that unfolded protein response (UPR) inhibitors in combination with standard chemotherapy may improve the effectiveness of antitumour therapies [103]. Hypoxia-inducing factor 1 α (HIF1 α) is known to be hyperactivated in TNBCs [104,30]. Genome-wide mapping of the XBP1 transcriptional regulatory network revealed that XBP1 drives TNBC tumorigenicity by assembling a transcriptional complex with HIF1 α that regulates the expression of HIF1 α targets via the recruitment of RNA polymerase II. Analysis of independent cohorts of patients with TNBC revealed a specific XBP1 gene expression signature that was highly correlated with HIF1 α and hypoxia-driven signatures and that strongly associated with poor prognosis. Recent findings suggest that XBP1 branch of the UPR has a key function in TNBC and indicate that targeting this pathway may offer alternative treatment strategies for this aggressive subtype of breast cancer.

Conclusion

This present review has provided insight into the roles of miRNA expressions and their validated targets in the identification and classification of breast cancer particularly TNBC in relation to metastases and overall survival rate. More so, it has provided knowledge into the risk factors, especially the KRAS oncogene and the place of Epithelial-to-Mesenchymal Transition (EMT) in the pathogenesis of TNBC. The few discussed treatments may be invaluable in the development of other therapeutic options in TNBC. These insights may provide future preventive measures, novel diagnostic tools and effective management scheme for all breast cancer subtypes.

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