Breast Cancer-Associated Fibroblasts

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Breast cancer-associated fibroblasts (BCAFs) are the CAFs present in breast cancers with genetic and phenotypic characteristics similar to CAFs. CAFs originate from a diverse range of cells, including endothelial cells, adipocytes, pericytes, and MSCs. Although CAFs were derived from endothelial cells and pericytes, the derivation was not tested in breast cancer models similar to other cancers. BCAFs have also been derived from adipocytes that lead to a desmoplastic microenvironment. BCAFs can originate from MSCs, which contribute to angiogenesis through up-regulation of clusterin leading to tumorigenesis. BCAFs possess the fibrillar collagen receptor, DDR2, which rearranges collagen fibers to develop an invasive and metastatic TME. Additionally, integrin α11 in BCAFs interacts with platelet-derived growth factor receptor beta (PDGFRβ) and promotes invasiveness by activating c-Jun N-terminal kinase (JNK) and producing a matricellular protein, tenascin C.

triple-negative breast cancer | breast cancer-associated fibroblasts | breast cancer associated fibroblasts | breast cancer | chemoresistance | nanotechnology | microRNA | antibodies

1. Free Small Molecules

1.1. TGF-β Inhibitors

TGF-β1 is a cytokine that binds to ligand binding receptors and recruited the receptors termed TGF-βRII and TGF-βRI, respectively [1][2]. TGF-β1′s engagement with TGF-βRII causes the recruitment and activation of the TGF-βRI, which phosphorylates the SMAD proteins (SMAD2 and 3). This pathway regulates the ECM genes to express ECM components, such as collagens and fibronectins, which produce dense fibrotic tissue [3].

Synthesized artesunate (ARS) and dihydroartemisinin (DHA) are derivatives of artemisinin extracted from Sweet Wormwood (*qinghao*), which have previously demonstrated antitumor activity in leukemia, colon cancer, fibrosarcoma, and breast cancer [4]. ARS and DHA negatively impacted some BCAFs in an orthotopic 4T1 model of mice by suppressing TGF-β signaling [5]. In this study, the first BCAFs were isolated from murine models of MMTV-PyMT to represent a subset of TNBC patients with luminal androgen receptor expression [6]. The BCAFs were then treated with ARS and DHA. Although there was no significant impact on cell viability compared to the control, the expression of CAF markers, including α-SMA, FAP and fibronectin, was significantly reduced ($p < 0.01$). Additionally, ARS and DHA were shown to repress the TGF-β signaling to inhibit BCAF activation and reduce tumor

growth and metastasis in vivo [5]. Significantly decreased TGF-β1 and phosphorylated SMAD3 levels showed that ARS and DHA were inhibiting the TGF-β signaling.

Pirfenidone is a TGF-β antagonist and has been approved for clinical use to treat idiopathic pulmonary fibrosis [7]. It has been effective as an antifibrotic agent in various preclinical studies with different conditions, such as nonalcoholic steatohepatitis and pancreatic cancer [8][9]. Takai et al. used pirfenidone to target BCAFs derived from syngeneic and xenograft models of TNBC [10]. Pirfenidone inhibited BCAF proliferation and fibrosis. It also caused apoptosis of both cancer cells and BCAFs. Furthermore, this group showed that pirfenidone inhibited fibrosis and TGF-β signaling but did not prevent the growth of TNBC tumors in vivo. The combination of pirfenidone with doxorubicin synergistically inhibited tumor growth and metastasis in the 4T1 syngeneic tumor model of TNBC. The strength of this study is that they isolated BCAFs from breast cancer patients and characterized them using Vim, FAP and the absence of an epithelial tumor marker, pan-cytokeratin [10]. One limitation of this study was that CAFs were injected along with cancer cells in mice, so the impact on the basal level of BCAFs in breast TME was not possible to be determined. Another limitation is that they did not explore the subpopulation of BCAFs that was impacted by the treatment.

Tranilast is an antihistamine drug and TGF-β inhibitor. This drug was shown to effectively target BCAFs in TNBC mice models [11]. Tranilast decreased ECM components and increased perfusion and infiltration of T cells. When combined with Doxil® (liposomal doxorubicin) to treat TNBC, it improved treatment efficacy, expression of immunostimulatory macrophage M1, and enhanced immune checkpoint blocking antibodies [11]. Another novel strategy used emodin (6-methyl-1,3,8-trihydroxyanthraquinone), which has demonstrated anti-inflammatory, antiviral, anticancer, and pro-apoptotic activities [12]. Hsu et al. extracted BCAF from tumor tissues of TNBC patients and examined the effects of BCAF conditioned medium on epithelial BT-20 breast cancer cells [13]. Emodin inhibited cell migration and EMT through TGF-β induced by BCAFs [13].

1.2. Dual Targeting Agents: Combined Anti-BCAF and Other Pharmacological Activity

Several anticancer agents have been found to have an impact on BCAFs. In one study, the BCAF-inhibitory potential of 138 compounds was estimated using the Cancer Genome Atlas and Genomics of Drug Sensitivity in Cancer databases of TNBC patients and associations with α-SMA expression. BCAFs have different expression levels of α-SMA (high and low) in different tumor models [14]. Embelin is a quinone derived from *Embelia ribes Burm* plants and one of the 24 agents that were estimated to have an impact on α-SMA levels [14][15]. Embelin has shown anticancer activity in a variety of cancers, such as oral squamous cell carcinoma and lung cancer [15]. Embelin was tested in two α-SMA classified tumor types, 4T1 (high α-SMA) and 4T07 tumor models (low α-SMA) [14]. Embelin's reduction of tumor volume was higher in 4T1 tumor (high α-SMA) than in 4T07 tumor (low α-SMA) [14]. It is likely that there are two populations of BCAF, however, further characterization of these populations is required.

Cisplatin is a DNA crosslinker, which is traditionally used for the treatment of testicular, ovarian, bladder, head and neck, lung, and breast cancer [16]. In a clinical trial, short-term cisplatin treatment was used as part of a combination therapeutic strategy with nivolumab to treat metastatic TNBC. Cisplatin caused up-regulation of immunogenic genes and increased the response rate to PD-1 blockade by altering the TME [17]. Balog et al. investigated the immunophenotype of the TNBC 4T1 mice model, as well as the tumor stroma following treatment with cisplatin. They observed that FAP proteolytic activity decreased as a result of cisplatin treatment [18]. It was speculated that the anticancer activity of cisplatin involved BCAFs inactivation [18]. However, further investigation is required to validate the study results, such as determining the expression of other BCAF markers, such as α-SMA and collagen.

BCAF secreted chemokine, CXCL12, binds to the CXCR4 chemokine receptor in cancer cells and regulates signaling pathways that allow growth, chemotherapy resistance, and metastasis [19][20][21]. Bicyclam AMD3100 is a CXCR4 antagonist and an approved drug for hematopoietic stem cells mobilization. It enables stem cell transplantation and is used for hematologic malignancy and other diseases, such as bone marrow failure and sickle cell disease [19]. In cocultures of TNBC cells and BCAFs (both 2D and 3D), AMD3100 normalized cancer cell growth to the level observed in cells without any CXCL12 signaling [22]. Furthermore, CXCL12 and CXCR4 signaling stimulated cell growth and invasion, which was prevented by AMD3100 [22].

Cabozantinib is an inhibitor of the tyrosine kinase receptor MET, which functions as an anticancer agent [23]. Hepatocyte growth factor (HGF) is secreted from CAFs at elevated levels and stimulates MET signaling [24]. Cabozantinib prevented invasion and growth of HGF-overexpressed TNBC cells (MET positive MDA-MB-231 and HCC70) co-cultured with BCAFs, which also had HGF overexpression. This treatment did not affect MET negative TNBC cell lines, which reflected the specificity of this inhibitor [24]. However, this strategy can only be used against certain subtypes of TNBC where MET overexpression exists and a subset of BCAFs that has HGF expression.

The Notch signaling pathway regulates cell–cell communication. Overexpression of Notch receptors is highly associated with the aggressive phenotype of TNBC, including invasiveness and resistance to chemotherapeutics [25]. Notch receptors can be blocked by a γ-secretase inhibitor, DAPT (N-[N-(3,5-difluorophenacetyl)-lalanyl]-S-phenylglycine t-butyl ester), which has shown activity against TNBC [25][26]. DAPT led to a reduced invasion of MDA-MB-231 co-cultured with CAFs [27]. It also inhibited CXCL8 (a pro-metastatic chemokine) in TNFα stimulated co-cultures of MDA-MB-231 and CAFs. The interaction between TNBC and CAFs amid the presence of TNFα resulted in increased migration and invasion. This was prevented by DAPT treatment through the inhibition of Notch signaling [27].

2. Nucleic Acid-Based Targeting Agents

MicroRNAs (miRNAs) are non-coding RNAs that regulate 30% of the gene expression of mRNA by binding to specific sites [28]. MiRNAs are aberrantly expressed in breast cancers compared to healthy breast tissue [29]. Significant deregulation of microRNA-21 (miR-21), miR-155, miR-145, and miR-125b has been discovered in

breast cancers [29]. The overexpression of these miRNAs plays a crucial role in tumor progression and invasion in breast cancer [29].

2.1. Small Molecules Targeting miRNAs

MiR-21 is abundantly expressed in breast cancers and is known to be associated with advanced disease and poor survival [30][31][32]. The Kang group confirmed that miR-21 is up-regulated in in vitro and orthotopic TNBC (MDA-MB-231) mice models [33]. This group corrected miR-21 expression using AC1MMYR2 (AMR), a miR-21 small molecule inhibitor, which also reduced tumor growth by reversing the transition of epithelial to mesenchymal cells [33]. They later demonstrated that miR-21 up-regulation was strongly associated with breast cancer lymph node metastasis. They also found that miR-21 activation is directly related to the metastatic effect of BCAFs through NF-κB axis [34]. Treatment with AMR decreased levels of α-SMA and FAP-α. Combination therapy using AMR and paclitaxel in an in vivo model reduced tumor growth and lung metastasis. This study shows that AMR effectively targets both α-SMA and FAP-α expressing BCAFs and TNBC depending on the presence of miR-21 [34].

Curcumin is the active compound in the *Curcuma longa* herb, which is more commonly known as turmeric [35]. It has been studied in various clinical trials due to its anticancer activities and high safety (up to 12 g/day) for human consumption [36][37]. The Aboussekhra laboratory determined that curcumin treatment upregulated p16 in BCAFs, which caused inactivation and senescence (growth arrest) of BCAFs [38]. Furthermore, curcumin inhibited the migration and invasion capabilities of MDA-MB-231 cultured with BCAF-conditioned medium and reduced BCAF secretion of SDF-1, IL-6, MMP-2, MMP-9, and TGF-β [38]. In a later study, the Aboussekhra group revealed that the up-regulation of p16 induced the miR-146b-5p expression, which led to reduced level of BCAF-secreted factors and prevented EMT, migration, and invasion of MDA-MB-231 [39].

2.2. Mimics and Anti-miRNAs

The TGF-β pseudoreceptor, bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI) are mimics of the TGF-β receptor without serine/threonine kinase domain that is necessary for TGF-β/SMAD signaling [40]. The transduction of cells with BAMBI using the lentiviral vector inhibited TGF-β/SMAD signaling [41]. The transformation of bone marrow mesenchymal stem cells (BM-MSCs) into CAFs has been demonstrated to occur in the presence of TGF-β and cancerous cells. BAMBI transduced mesenchymal cells that were co-inoculated with MDA-MB-231 cells and caused the inhibition of tumor growth and metastasis, while the parent mesenchymal cells promoted tumor growth and metastasis. Furthermore, BAMBI treatment prevented BCAFs differentiation from BM-MSCs [41].

The down-regulation of a tumor suppressor miRNA, called let-7b, caused the activation of BCAFs [42]. In this study, BCAF activation was demonstrated by the presence of α-SMA, SDF-1 and TGF-β1. The BCAF activation occurred through IL-6 induction, which led to a high level of IL-8 expression, which subsequently promoted the EMT in cancer cells. Additionally, deregulation of let-7b was shown to enhance the migration and invasion capacity of BCAFs and tumor growth. Let-7b mimics restored the levels of let-7b in BCAFs and reduced the pro-tumorigenic

functions of BCAFs. Furthermore, BCAF inactivation was confirmed by demonstrating decreased levels of α-SMA, IL-8 and TGF-β1 as well as CXCL12 (also known as SDF-1). Let-7b mimics inhibited migration and invasion in TNBC cells (MDA-MB-231) in vitro [42]. Further studies are required to establish that let-7b mimics are effective against different sub-groups of BCAFs and in TNBC in vivo models.

Exosomes are vesicles of approximately 40 to 100 nm in size and are used as intercellular communication mediators in cancer [43]. Anti-miRs were utilized to inhibit the function of exosomes secreted by BCAFs [44]. These exosomes overexpressed miR-21, miR-143, and miR-378e, which increased stemness, EMT phenotype, and proliferation in TNBC. MDA-MB-231 cancer cells were transfected with anti-miR-21, -143, and -378e, which inhibited the effects of BCAFs' exosomes [44]. However, the group only investigated the effect of anti-miRs in a transfected breast cancer cell line (T-47D) in vitro. The efficacy of anti-miRs needs to be confirmed in TNBC models in the future.

2.3. Aptamers

Aptamers are single-stranded DNA and RNA oligonucleotides that can be designed into structures that target small molecules, proteins, and live cells [45]. These aptamers are generally produced through the systematic evolution of ligands through the exponential enrichment process (SELEX), where the oligonucleotides are projected to iterative partitioning to form the desired structure [46][47].

Locked nucleic acid (LNA)-i-miR-221 oligonucleotide treatment significantly inhibited BCAF and TNBC (MDA-MB-231) cell growth and migration [48]. LNA-i-miR-221 prevented the function of miR-221 that promotes cell growth and migration. The mechanism of action of LNA-i-miR-221 was revealed to be through down-regulation of the expression of the A20 ubiquitin editing enzyme and increased transcription factor c-Rel and connective tissue growth factor. The BCAFs used in this study were derived from mammary ductal carcinoma mastectomies (the TNBC status of the mastectomies was not confirmed) [48]. Further studies should investigate the efficacy of LNA-i-miR-221 in a coculture of TNBC cancer and BCAF cells and in in vivo models of TNBC that would more accurately demonstrate the efficacy of the agent in tumor growth and progression.

MiR-9 has been proven to be highly up-regulated in primary TNBC BCAFs compared to normal fibroblasts [49]. MiR-9 transformed normal fibroblasts into CAFs, which then promoted migration, invasion, and motility. This was effectively inhibited using locked nucleic acid targeted against miR-9 (LNA-9), which reversed the migration, invasion, and motility phenotype in BCAF. It was speculated that TNBC cells and BCAFs secrete exosomes containing miR-9, which increase the motility of TNBC cells and BCAFs [49]. Their study design consisted of using immortalized normal fibroblasts transfected with miR-9 to represent CAFs and injecting them alongside TNBC cells (MDA-MB-468) into SCID mice to demonstrate BCAF-promoted tumorigenesis [49]. This could have been improved by using TNBC BCAFs because it could mimic the functions of BCAFs more closely compared to immortalized normal fibroblasts transfected with miR-9. Furthermore, the sub-populations impacted by this treatment should also be determined.

BM-MSCs have the ability to differentiate into other cell types, such as CAFs and tumor-associated macrophages [50]. The platelet-derived growth factor receptor (PDGFRβ) has a significant role in MSC recruitment to the tumor site [51]. Furthermore, the basic fibroblast growth factor and platelet-derived growth factor BB support the function of bone marrow in the dissemination of breast cancer cells [52]. The nuclease resistant aptamer called Gint4.T is an RNA-based oligonucleotide that specifically binds with high affinity to PDGFRβ and blocks its activity [53]. Cocultures of BM-MSC and TNBC cells resulted in increased mRNA levels of α-SMA, FAP and FSP-1, which confirmed that BM-MSC can transdifferentiate into CAF-like cells [54]. The Gint4.T inhibited BCAF formation from BM-MSC and the metastatic ability of the BM-MSC-induced TNBC (MDA-MB-231) tumor in vivo [54].

Although this approach may be potentially promising, the regulation of miRNAs is complex, as miRNA may have various functions that can complicate the further development of RNA-targeting agents and their clinical translation [55]. Only one aptamer, pegaptanib (Macugen®), has been approved in the last 3 decades since its discovery due to challenges such as rapid excretion and low in vivo binding affinity [56][57]. Only three oligonucleotides have been in clinical trials to date; two were terminated in early phase trials and none have yet been approved for cancer treatment [58][59]. Thus, this field of research requires further preclinical validation prior to clinical translation.

3. Antibodies and Proteins

Antibodies and protein-based agents have been used to inhibit certain functions of BCAFs in TNBC [60][61]. The major concern with traditional chemotherapy drugs for TNBC is the lack of specificity and limited efficacy [62]. Therefore, as antibodies are highly specific and bind only to their target receptor, they are used in the development of targeted anti-BCAF agents and have high potential for the treatment of aggressive TNBC.

3.1. Antibodies

BCAFs induced chemotherapy resistance through the activation of interferons, such as Type I and II [60]. The interferon signaling of BCAFs was demonstrated by communication between interferon β1 in BCAFs and the MX1 protein in TNBC cells. Type I and II interferon signaling blocking antibodies reversed BCAF-induced chemoresistance in some TNBC cells (MDA-MB-231 and MDA-MD-157). However, this effect was not present in TNBC MDA-MB-468 cells and the different responses of TNBC cells were attributed to the TNBC subtype [60]. Herschkowitz et al. identified a molecular subtype of breast cancer cells termed as ‘claudin-low’, which was characterized by minimal expression of genes that function in epithelial cell–cell adhesion and tight junctions, such as E-cadherin and claudin 3 [63]. This claudin-low subgroup has been correlated with a poor prognosis and chemotherapy resistance [64][65]. Blocking antibodies against Type I and II interferon signaling were only effective in claudin-low cells (MDA-MB-231 and MDA-MD157) and not in the claudin-high TNBC cell line (MDA-MB-468) [60]. There is a potential for the clinical translation of this research after further investigation in animal models. This is due to the availability of clinically approved interferon receptor blockers, such as anifrolumab (a monoclonal antibody) and inhibitors of the interferon signaling pathway, such as ruxolitinib (a small molecule) [66][67].

3.2. Proteins

Integrin αvβ3, a transmembrane protein, is highly expressed in CAFs [68]. A new synthetic protein, ProAgio, was developed to target the integrin αvβ3, which caused apoptosis through the recruitment of Caspase-8 [61]. ProAgio successfully induced apoptosis in integrin αvβ3 containing TNBC BCAFs and angiogenic endothelial cells. BCAFs depletion due to ProAgio resulted in reduced intratumoral collagen, growth factors secreted by BCAF, resistance to apoptosis, and proliferation of cancer cells. Furthermore, ProAgio suppressed tumor growth and improved survival in the TNBC (MDA-MB-231) xenograft model in mice [61].

3.3. Antibody–Drug Conjugates

The development of antibody–drug conjugates (ADCs) has been another approach to eliminating TNBC BCAFs [69]. Eribulin mesylate is a fully synthetic macrocyclic ketone that is clinically approved for metastatic breast cancer in the United States and is currently used in more than 60 countries worldwide [69][70]. ADCs are developed to address the limited specificity problem of traditional chemotherapy, where the antibody will safely deliver the drug to the target site [69]. Various eribulin-based ADCs are under investigation. Farletuzumab is a monoclonal antibody targeting the folate receptor alpha (FRA) and has been in clinical trials for the treatment of epithelial ovarian cancer [71]. FRA is abundantly expressed in TNBC and is correlated with a poor prognosis [72][73]. Furuuchi et al. developed MORAb-202 antibody–drug conjugate that is composed of eribulin mesylate and farletuzumab coupled through a cathepsin B– cleavable linker. MORAb-202 showed an FRA receptor-mediated antitumor activity and reduced the expression α-SMA of CAFs compared to the control. Furthermore, in a patient-derived xenograft TNBC model, BCAF was demonstrated to be affected by MORAb-202 through the bystander effect. This led to enhanced tumor suppression and sensitization to therapy [69].

4. Nanoparticles

Nanoparticles consist of multiple components that allow drugs or compounds to be delivered to the site of interest and unleash a certain effect [74]. They have the ability to be easily tuned and functionalized for active targeting, deliver a large quantity of therapeutics to tumor cells, and provide passive targeting via the enhanced permeability and retention (EPR) effect to reach tumor tissue (by extravasation through leaky tumor vessels). Furthermore, nanoparticles can be engineered to deliver a consistent amount of payload and release therapeutic agents with a high level of control [75]. Various nanoparticles were designed to target BCAFs in TNBC.

4.1. Gold Nanoparticles

Gold nanoparticles (15 nm diameter) targeting αvβ3 integrins demonstrated high efficacy in uptake and retention in MDA-MB-231 and CAFs [76]. These gold nanoparticles were functionalized with polyethylene glycol and an integrin-binding peptide, arginine-glycine-aspartate (RGD), which targets cells with αvβ3 integrin to improve tumor cell uptake [77]. The uptake of gold nanoparticles was approximately six and 12 times higher in MDA-MB-231 cells and CAFs, respectively, in comparison to fibroblasts [76]. The retention period was also 30% longer in TNBC cells and CAFs compared to fibroblasts. This delivery system could achieve optimal drug delivery to cancerous cells and CAFs [76]. In this study, CAFs were derived from melanoma tumors, and nanoparticles were tested in monocultures

of CAFs and breast cancer cells. However, to better elucidate the effects of gold nanoparticles in TNBC with respect to BCAF, a model involving the coculture of BCAFs and MDA-MB-231 cells would be more beneficial and characterizing the subset of BCAFs that are impacted.

4.2. Lipid-Based Nanoparticles

An indirect method of targeting BCAFs is by suppressing their function and blocking their secreted factors [74]. BCAFs produce tenascin C (TN-C), an extracellular glycoprotein [78][79]. Li et al. developed lipid-based nanoparticles (mixture of sulfatide and perfluorooctylbromide) nanoparticles. Sulfatide can bind to several ECM glycoproteins, including TN-C, and can be readily incorporated into the structure of lipid nanoparticles [80]. These nanoparticles were developed to selectively deliver paclitaxel to the tumor microenvironment. Paclitaxel-loaded targeted nanoparticles resulted in higher cytotoxicity and tumor inhibition than untargeted nanoparticles in the syngeneic EMT6 TNBC model of mice. This study evidenced that indirect targeting of BCAFs through TN-C can enhance the activity of chemotherapeutic agents [80].

Interleukin 10 (IL-10) that is secreted by BCAFs and is highly expressed in TNBC, contributes to immunosuppressive TME that results in immunotherapy resistance [81][82]. CXCL12 is also secreted by BCAFs and directly promotes tumor growth [20]. Another indirect BCAF targeting approach used nanoparticles loaded with IL10 and CXCL12 trap genes [83]. This nano-delivery system consisted of liposome-protamine-DNA nanoparticles, genes encoding the IL-10 or CXCL12 protein trap, and polyethylene glycol. This IL-10 trap-based nanotechnology significantly inhibited tumor growth and improved survival in an orthotopic TNBC 4T1 model. Combination treatment using both trap genes suppressed immunosuppressive cells such as PD-L1+ cells and M2 macrophages within the tumor [83].

Silybin or silibinin is a flavonolignan of *Silybum marianum*, a medicinal plant that has been used for thousands of years for liver diseases and is known for its antioxidant, anti-inflammatory, and antifibrotic power [84]. Due to its known activity in the treatment of liver fibrosis, Wu et al. investigated the effects of silybin on the eradication of CAFs in the TNBC 4T1 model [85]. Nanoliposomes were fabricated as carriers of silybin to increase its stability and bioavailability. They determined that α-SMA expression was significantly reduced, and immune responses improved through increased IFN-γ, IL-12 and cytotoxic T cells. In vivo, combination treatment with liposomal doxorubicin led to immunogenic tumor apoptosis and prolonged survival. The combination of liposomal silybin with liposomal doxorubicin reduced tumorigenesis and further prolonged the survival of mice [85].

Marimastat is an enzyme inhibitor that is active against gelatinases, collagenases, and metalloproteinases [74]. Hybrid nanoparticles (HNPs) comprising marimastat-loaded thermosensitive liposomes together with the hyaluronic acid-paclitaxel prodrug were designed as a dual-targeting system to deliver agents to the tumor microenvironment and cancerous cells. HNPs released marimastat and the hyaluronic acid-paclitaxel prodrug under mild hyperthermic conditions in the tumor microenvironment [86]. The hyaluronic acid-paclitaxel prodrug impeded the survival of cancerous cells and marimastat decreased TGF-β1, TN-C and α-SMA expression, metalloproteinase

activity, and cancer cell migration. Treatment with HNPs in the murine model of TNBC resulted in significant inhibition of tumor growth, metastasis, angiogenesis, and negatively affected some BCAFs [86].

Puerarin is an isoflavone derived from the kudzu root with potential applications in the reduction of blood pressure and myocardial oxygen consumption and in ischemia–reperfusion injury [87]. Puerarin negatively regulated reactive oxygen species production in CAFs [88]. A puerarin nanoemulsion (nano-puerarin) was developed using lecithin and aminoethyl anisamide as the targeting ligand for the sigma receptor, which is abundantly expressed on CAFs and cancerous cells. Additionally, it significantly decreased α-SMA in the murine 4T1 TNBC model compared to the untreated control. This led to a reduction in collagen, BCAF secreted immune suppressive cytokines such as IL-6 and IL-10, and an increase in T cells (CD8+ and CD4+) in the tumor. When nano-puerarin was used in combination with paclitaxel nanoparticles formulated using a nanopolymer system, it increased the efficacy of nano-paclitaxel. Nano-puerarin deactivated desmoplastic TME by decreasing α-SMA positive BCAFs in the TNBC tumor [88].

4.3. Polymeric and Hydrogel-Based Nanoparticles

Angiotensin receptors function by binding to the angiotensin II hormone and play crucial roles in the human body, including renal regulation, cell growth, and survival [89]. Angiotensin receptor blockers (ARBs) are antagonists such as valsartan and losartan, which are clinically used to treat high blood pressure [89]. Many ARBs, including valsartan and losartan, are based on a biphenyl tetrazolo structure [90]. In addition to these, ARBs have also been shown to have CAF reprogramming effects and reduce α-SMA+ CAF levels [91]. However, an important challenge is that ARBs can cause hypotension. Hence, novel delivery systems are being demanded to reduce their on-target side effects in cancer application. An ARB nanoconjugate with valsartan attached to polyacetal (1,1,1-Tris(hydroxymethyl)ethane, di(ethylene glycol) divinyl ether and polyethylene glycol) [92]. These nanoconjugates were designed to overcome the hypotension side effect generated by ARBs. Thus, the ARBs were chemically linked with an acid-degradable polymer that is sensitive to the pH of the tumor. These ARB nanoconjugates were examined in murine models, where they accumulated in high concentrations, were active in tumors, and remained inactive in the circulation.

Losartan is an angiotensin inhibitor that has been approved as a hypertension management medicine [93][94]. It also demonstrated efficacy in reducing cardiac and renal fibrosis [95][96]. In some studies, losartan has inhibited collagen, hyaluronan production, and profibrotic signaling of TGF-β1 in syngeneic TNBC models of mice [91]. There is substantial evidence that losartan can reduce collagen I production and solid stress and negatively impact some BCAFs [91][97]. The peptide (C16-GNNQQNYKD-OH) encapsulated losartan in a hydrogel form. Losartan-loaded nanohydrogels were used to target BCAFs in the murine TNBC model (4T1) [98]. This nanoparticle was delivered locally through intratumoral injection and reduced the expression of α-SMA+ BCAFs and collagen. In addition, it improved the efficacy of chemotherapy (Doxil®), suppressed tumor growth, and metastasis [98].

4.4. Nano-Ferritins

Ferritin nanocages (10 to 12 nm diameter) are composed of a protein that uses Fe_2O_3 in its building blocks, storing iron in its cavity [99]. Holoferritine (H-ferritin) is a type of ferritin that can change iron states (oxidize Fe^{2+} to Fe^{3+})

[100]. Sitia et al. targeted BCAFs directly using the H-ferritin nanocage delivery system [101]. The H-ferritin nanocages were conjugated with FAP antibody fragments. Since FAP is expressed on the surface of BCAFs, this enabled the functionalized H-ferritin nanocages to bind specifically to the BCAFs. The conjugated H-ferritin nanocages were loaded with navitoclax, a B-cell lymphoma 2 (BCL-2) inhibitor that acts by inducing apoptosis. Conjugated H-ferritin nanocages loaded with navitoclax (HNav-FAP) induced significantly higher cytotoxicity in BCAFs compared to untargeted H-ferritin nanocages.

Photodynamic therapy is based on the use of a non-toxic dose of light in cells containing light-sensitive molecules (photosensitizer) [102]. Photodynamic therapy is used as an anticancer therapeutic approach without targeting photosensitizer molecules. Rather, by eliminating cancerous cells by creating reactive oxygen species or damaging the vasculature [103]. Ferritin nanocages with anti-FAP single chain variable fragment antibody and a photosensitizer were utilized to target BCAFs in the syngeneic 4T1 TNBC in vivo model. FAP expressing BCAFs were selectively eradicated due to the localized photoirradiation. Additionally, CXCL12 secretion by BCAFs was reduced, which improved antitumor immunity through increasing CD8+ T-cell infiltration [102]. A similar study was conducted using the FAP-targeted photosensitizer described above to increase the penetration of quantum dots [104].

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