Cancer Reports and Reviews

Mini Review ISSN: 2513-9290

Cancer stem cells and breast cancer

Hyder SM* and Slight SH

Department of Biomedical Sciences and the Dalton Cardiovascular Research Center, University of Missouri-Columbia, 134 Research Park Drive, Columbia, MO 65211, USA

Breast cancer is one of the leading causes of death in women, both globally and in the United States. In the US over 40,000 women die from the disease annually [1]. Although breast cancer is an extremely heterogeneous disease that may be characterized by the presence or absence of various phenotypic markers, the initiation, proliferation and ultimate metastasis of breast tumors is dependent on pluripotent cancer stem cells (CSCs); undifferentiated CSCs can self-renew and become differentiated into a variety of specialized cell types [2]. Compared with the majority of cells within a tumor, CSCs are more resistant to established methods of chemotherapy, including radio-, chemo- and hormone treatments. When transplanted into an animal model, CSCs have the capacity to seed new tumors. CSCs typically exhibit a specific phenotypic signature of $CD24^{low/-}$, $CD44^{high}$, ALDH (aldehyde dehydrogenase) [3]. The cell surface glycoprotein, CD44 plays a role in cell/cell communication, cell adhesion and migration. Since CSCs resist conventional breast cancer therapies, there is an urgent need to develop specialized treatments to decrease their population and thereby reduce metastasis.

There is overwhelming evidence that women undergoing hormone replacement therapy (HRT) containing a progestin component have an elevated risk of developing breast cancer [4,5]. Studies *in vitro* and *in vivo*, show that both progesterone, and a variety of synthetic progestins that are widely used clinically, stimulate the production of vascular endothelial growth factor (VEGF) by cancer cells, leading to angiogenesis and cancer metastasis [6,7]. Such treatments also enrich the pool of CSCs within tumors. Medroxyprogesterone acetate (MPA), a synthetic progestin used in HRT, induces different variants of CD44 in T47-D human breast cancer cells. MPA also increases ALDH enzyme activity and promotes the formation of clumps, or mammospheres, another trait of CSCs. MPA induction of CD44 and elevation of ALDH activity are both dependent on MPA binding to the progesterone receptor (PR), since both are inhibited by the PR antagonist RU-486 [8]. Variations of CD44 protein are produced through alternative splicing. Treatment of T47-D cells with MPA specifically induces two of these variants, CD44v3 and CD44v6, the latter of which plays a role in extracellular matrix degradation, invasion and metastasis.

Tumors that resist chemotherapy often display abnormal regulation of cholesterol homeostasis. Studies in our laboratory show that the compound RO 48-8071 (RO), which inhibits cholesterol biosynthesis, may be a viable agent for suppressing the CSC content of breast tumors and thereby preventing metastasis to distant organs [9]. RO specifically inhibits the enzyme 2,3-oxidosqualene cyclase (OSC), which acts downstream of HMG-CoA reductase (target of statins) in the biosynthetic pathway leading to cholesterol. We found that RO reduced MPA-induced CD44 protein expression in two different human breast cancer cell lines, T47-D and BT-474 cells. Furthermore, while not affecting expression of PR mRNA, RO reduced levels of both isoforms of the receptor, PR-A and PR-B in the two cell lines. Exposure of T47-D cells to RO abolished their ability to form mammospheres. As well as influencing CSCs, RO exerts several other anti-cancer effects, including reducing estrogen receptor-alpha (ER)α, which promotes breast cancer cell proliferation [10]. However, its ability to reduce levels of functional PR and disrupt the expression of PR-target genes such as WNT, thereby blocking the Wnt/β-catenin signalling pathway, is of particular interest here, since by so doing, RO inhibits the CSC-like phenotype and quashes metastasis.

Although most diagnosed breast cancers are hormone-dependent, a significant proportion are designated triple-negative breast cancer (TNBC), so named because they do not express PR, ER and human epidermal growth factor receptor (HER2/neu). Since they lack hormone receptors, TNBCs do not respond to progestins or estrogen. They are unresponsive to conventional therapies, including those that are used to block PR and ER and as a consequence TNBCs grow and metastasize aggressively. Women with TNBC have a poor prognosis and invariably succumb to the disease. The majority of triple-negative tumors express a defective mutant form of p53 tumor suppressor protein (mtp53), which, unlike its wild-type counterpart (wtp53), fails to promote cell-cycle arrest and apoptosis and permits unfettered VEGF-dependent angiogenesis [11,12]. Studies show that TNBC cells exhibit characteristics common to CSCs (CD24low/-, CD44high, ALDH) and readily form mammospheres [13,14]. Within TNBC cells and CSCs exists a complex system of self-renewal signalling pathways (SRSPs) that depend on key mechanisms, including STAT3, SRC kinase and the aforementioned Wnt/β-catenin signalling. TNBC cells utilize the glycolytic pathway and oxidative phosphorylation is an essential component of increased resistance to chemotherapy. Fatty acid oxidation satisfies the increased energy requirements of these cells, which possess a high degree of metabolic plasticity, another reason TNBC cells are extremely difficult to treat with conventional methods.

As bleak as the outlook once was for women afflicted with TNBC, there are a number of ongoing preclinical and clinical trials aimed at targeting specific metabolic pathways within TNBC stem cells [15]. Furthermore, we recently showed, in a mouse model, that conversion of mtp53 back into active wtp53 by the small molecule drug APR-246, inhibits metastasis of human TNBC cells to the lungs. Exposure of MDA-MB-231 and MDA-MB-435 TNBC cells to APR-246 inhibits

Correspondence to: Salman M Hyder, Department of Biomedical Sciences and the Dalton Cardiovascular Research Center, University of Missouri-Columbia, 134 Research Park Drive, Columbia, MO 65211, USA, Tel: +1 5738821261, E-mail: hyders@missouri.edu

Received: February 29, 2020; **Accepted:** : March 11, 2020 **Published:** March 16, 2020

both ALDH activity and mammosphere formation, suggesting that this agent might be used to reduce the population of CSCs in TNBC [16]. Metformin, a glycolysis inhibitor used principally to treat type 2 diabetes, suppresses CSCs and diminishes tumor-initiating potential [17]. Benserazide, which is used to treat Parkinson's disease, also shows promise in this regard [18]. Disrupting oxidative phosphorylation is a further means by which the metabolic plasticity of CSCs might be targeted pharmacologically. IACS-010759 inhibits the mitochondrial electron transport chain complex I and is being evaluated for its ability to inhibit the growth of TNBC cells [19]. Fatty acid oxidation is elevated in CSCs compared with non CSCs. Treatment of CSCs with etomoxir, which irreversibly inhibits the mitochondrial membrane enzyme carnitine palmitoyltransferase-1 (CPT-1) and disrupts ATP production from the oxidation of fatty acids, significantly decreases CSC viability, particularly in cells with high levels of CPT-1 activity [20]. Furthermore, etomoxir diminishes tumorigenesis in mouse models of TNBC. Unfortunately, although etomoxir has been used to treat diabetes and heart failure, it is associated with serious side effects, including liver toxicity. Perhexiline, which also inhibits CPT-1 and has undergone clinical trials to test its efficacy against angina, similarly reduces the CSC population and inhibits tumor growth [21], though it too has significant side-effects. Palbociclib (trade-name Ibrance) recently received FDA approval for treating HR+ HER2- metastatic breast cancer [22]. Palbociclib is typically taken in conjunction with an aromatase inhibitor. It acts by inhibiting cyclin-dependent kinases CDK4 and CDK6, thereby disrupting self-renewal of breast CSCs [23].

The effectiveness of certain naturally-occurring plant compounds, or nutraceuticals, against a variety of cancers, including TNBC, is an exciting prospect. Unlike most chemotherapeutic drugs used in clinical practice, such agents are generally non-toxic. We have examined the anti-tumor properties of a number of such compounds, including curcumin, apigenin and luteolin [24-26]. Using a mouse xenograft model, we showed that luteolin, a flavonoid found in many plants, including broccoli and celery, reduced the ability of MDA-MB-435 and MDA-MB-231 (4175) LM2 TNBC cells to metastasize to the lungs [26]. Furthermore, luteolin, even at relatively low levels, inhibited VEGF secretion by MDA-MB-231 LM2 cells, suggesting that it interferes with the angiogenic component of metastasis. Likewise, extract of blueberries has been shown to inhibit the proliferation of MDA-MB 231 cells, increase apoptosis and reduce TNBC metastasis in a mouse model [27]. Considering the aforementioned characteristics common to TNBC and CSCs, it is not unreasonable to propose that agents such as luteolin might be expected to exert inhibitory effects on the CSC population of a tumor. Studies along similar lines as those conducted using natural nutraceuticals and TNBC cells are necessary if we are to harness the potential of naturally-occurring non-toxic dietary compounds in the fight against cancer.

The population of CSCs within a breast tumor undoubtedly plays a significant role in determining the level of malignancy, whether the cancer is hormone-dependent or of the triple-negative variety. The degree of "stemness" largely dictates how a cancer might respond to chemotherapy, whether it develops resistance to anti-hormone treatment, and its potential to metastasize to different sites. Although breast cancer treatments have improved markedly in recent years, there is an urgent need for new therapies that target CSCs and improve the prognosis of women, both with TNBC, and hormone-responsive breast cancer. While the development of targeted drug therapies by the pharmaceutical industry will undoubtedly continue to be important in the battle against breast cancer, there is an increasing awareness of the disease-fighting potential of naturally-occurring plant compounds. Flavonoids and other bioactive substances that interfere with different metabolic processes occurring within breast cancer cells might be administered either alone, or in conjunction with conventional chemotherapeutic regimens. The concomitant use of non-toxic nutraceuticals could lower the needed dose of toxic anti-tumor drugs and thereby improve patient quality of life. The ability of nutraceuticals to suppress breast cancer stem cells therefore warrants further investigation.

Acknowledgements

Studies in the author's laboratory were supported by awards from the NIH, DOD, Susan Komen for Cure and peer-reviewed grant programs from the University of Missouri College of Veterinary Medicine. Funds provided by the Zalk Missouri Professorship to SMH also supported these studies. We would like to recognize Dr. Yayun Liang, and many students and post-doctoral fellows for their dedication and hard work in successfully carrying out these investigations, and want to express our thanks to our various collaborators for their invaluable expertise.

References

1. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics. *CA Cancer J Clin* 70: 7-30.
2. Dick JE (2008) Stem cell concepts renew cancer research. *Blood* 112: 4793-4807. [Crossref]
3. Ajani JA, Song S, Hochster HS, Steinberg IB (2015) Cancer stem cells: the promise and the potential. *Semin Oncol* 42: 1-17. [Crossref]
4. Ross RK, Paganini-Hill A, Wan PC, Pike MC (2000) Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. *J Natl Cancer Inst* 92: 328-332. [Crossref]
5. Beral V, Million Women Study Collaborators (2003) Breast cancer and hormone-replacement therapy in the million women study. *Lancet* 362: 419-427. [Crossref]
6. Liang Y, Hyder SM (2005) Proliferation of endothelial and tumor epithelial cells by progestin-induced vascular endothelial growth factor from human breast cancer cells: paracrine and autocrine effects. *Endocrinology* 146: 3632-3641.
7. Benakanakere I, Besch-Williford C, Schnell J, Brandt S, Ellersieck MR, et al. (2006) Natural and synthetic progestins accelerate 7,12-Dimethylbenz[*a*] Anthracene-initiated mammary tumors and increase angiogenesis in Sprague-Dawley rats. *Clin Cancer Res* 12: 4062-4071.
8. Goyette S, Liang Y, Mafuvadze B, Cook MT, Hyder SM, et al. (2017) Natural and synthetic progestins enrich cancer stem cell-like cells in hormone-responsive human breast cancer cell populations in vitro. *Breast Cancer: Targets and Therapy* 9: 347-357.
9. Liang Y, Goyette S, Hyder SM (2017) Cholesterol biosynthesis inhibitor RO 48-8071 reduces progesterone receptor expression and inhibits progestin-dependent stem cell-like cell growth in hormone-dependent human breast cancer cells. *Breast Cancer: Targets and Therapy* 9: 487-494.
10. Liang Y, Besch-Williford C, Aebi JD, Mafuvadze B, Hyder SM, et al. (2014) Cholesterol biosynthesis inhibitors as potent novel anti-cancer agents: suppression of hormone-dependent breast cancer by the oxidosqualene cyclase inhibitor RO 48-8071. *Brest Cancer Res Treat* 146: 51-62.
11. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumors. *Nature* 490: 61-70. [Crossref]
12. Walerych D, Napoli M, Collavin L, Del Sal G (2012) The rebel angel: mutant p53 as the driving oncogene in breast cancer. *Carcinogenesis* 33: 2007-2017.
13. Li H, Ma F, Wang H, Lin C, Fan Y, et al. (2013) Stem cell marker aldehyde dehydrogenase 1 (ALDH1)-expressing cells are enriched in triple-negative breast cancer. *Int J Biol Mark* 28: 357-364.
14. Ma F, Li H, Wang H, Shi X, Fan Y, et al. (2014) Enriched $CD44^+/CD24^-$ population drives the aggressive phenotypes presented in triple-negative breast cancer (TNBC). *Cancer Lett* 353: 153-159.
15. Park SY, Choi JH, Nam JS (2019) Targeting cancer stem cells in triple-negative breast cancer. *Cancers* 11: 965.

16. Liang Y, Besch-Williford C, Cook MT, Belenchia A, Hyder SM, et al. (2019) APR-246 alone and in combination with a phosphatidylserine-targeting antibody inhibits lung metastasis of human triple-negative breast cancer cells in nude mice. *Breast Cancer: Targets and Therapy* 11: 249-259.

17. Wahdan-Alaswad R, Edgerton S, Salem H, Thor A (2018) Metformin targets glucose metabolism in triple negative breast cancer. *J Oncol Transl Res* 4: 129.

18. Li W, Zheng M, Wu S, Gao S, Yang M, et al. (2017) Benserazide, a dopadecarboxylase inhibitor, suppresses tumor growth by targeting hexokinase 2. *J Exp Clin Cancer Res* 36: 58.

19. Molina JR, Sun Y, Protopopova M, Gera S, Bandi M, et al. (2018) An inhibitor of oxidative phosphorylation exploits cancer vulnerability. *Nat Med* 24: 1036-1046.

20. Camarda R, Zhou Z, Kohnz RA, Balakrishnan S, Mahieu C, et al. (2016) Inhibition of fatty acid oxidation as a therapy for MYC-overexpressing triple-negative breast cancer. *Nat Med* 22: 427-432.

21. Wang T, Fahrmann JF, Lee H, Li YJ, Tripathi SC, et al. (2018) JAK/STAT3-regulated fatty acid β-oxidation is critical for breast cancer stem cell self-renewal and chemoresistance. *Cell Metab* 27: 136-150.

22. Lathia J, Liu H, Matei D (2020) The clinical impact of cancer stem cells. *The Oncologist* 25: 123-131.

23. Brooks MD, Burness ML, Wicha MS (2015) Therapeutic implications of cellular heterogeneity and plasticity in breast cancer. *Cell Stem Cell* 17: 260-271.

24. Carroll CE, Benakanakere I, Besch-Williford C, Ellersieck MR, Hyder SM (2010) Curcumin delays development of MPA-accelerated DMBA-induced mammary tumors. *Menopause* 17: 178-184.

25. Mafuvadze B, Cook M, Xu Z, Besch-Williford CL, Hyder SM (2013) Effects of dietary apigenin on tumor latency, incidence and multiplicity in a medroxyprogesterone Acetate-Accelerated 7,12-Dimethylbenz(a)anthracene- Induced breast cancer model. *Nutr Cancer* 65: 1184-1191.

26. Cook MT, Liang Y, Besch-Williford C, Hyder SM (2017) Luteolin inhibits lung metastasis, cell migration, and viability of triple-negative breast cancer cells. *Breast Cancer: Targets and Therapy* 9: 9-19. [Crossref]

27. Adams LS, Kanaya N, Phung S, Liu Z, Chen S (2011) Whole blueberry powder modulated the growth and metastasis of MDA-MB-231 triple negative breast tumors in nude mice. *J Nutr* 141: 1805-1812. [Crossref]