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The role of radiotherapy-resistant stem cells in breast cancer recurrence

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Although the effectiveness of anti cancer therapies has improved dramatically, breast cancer (BC) remains the second leading cause for cancer-related death among women in the western world [1]. The observed improvement in the survival rate and remission for primary tumors are essentially related to the advances in early diagnosis with more sensitive imaging technology [2,3]. However, the death rate remains unacceptably high for BC patients due to hard-to-treat metastatic and recurrent tumors, necessitating new, effective approaches and anticancer agents. Accumulating evidence suggests that cancer stem cells (CSCs), which are present in many cancers including BC, mediate tumor metastasis and contribute to relapse due to their resistance to current conventional therapies [4,5]. These unique cells possess stem cell-like characteristics, such as the capacity of self-renewal, which makes the tumor capable of regenerating its entire bulk. CSCs are resistant to proapoptotic factors, rendering them a formidable adversary to anticancer agents. In part, this is related to their quiescence capacity, which holds them in a standby mode in their niche microenvironment, sheltering them from radiation and other anticancer agents, since these agents are effective only on highly proliferative cells [6,7]. In addition to their powerful DNA repair machinery, breast CSCs (BCSCs) express ALDH, which is suspected to play a part in their death-resistance phenotype by being involved with cell detoxification machinery [8,9]. Thus, BCSCs represent a major challenge in the battle against BC, and an opportunity to develop more effective target to treat metastatic lesions.

Radiotherapy (RT), a recognized treatment modality for BC patients [10,11], is also considered appropriate for patients with high risk of recurrence [12]. Although substantial benefits are achievable with this treatment, especially for ductal carcinoma and early invasive cancer, advanced invasive tumors can develop radioresistance, and the molecular mechanisms involved are yet unknown. Recently, tumor radio-resistance has been linked to the activation of NF- κ B, a ubiquitous transcriptional factor in mammalian cells [13]. The induction of NF- κ B is associated with the activation of an array of antiapoptotic genes, such as *MnSOD* and *MKPI*, which scavenge damaging free radicals and inhibit the apoptosis

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signaling pathway (JNK) [14,15]. Interestingly, the NF- κ B-regulated genes act partially on the apoptosis mechanism mediated by mitochondria [16,17], since some of those proteins are detected in the mitochondria in response to irradiation. These data suggest that mitochondria metabolism is involved in the development of radioresistant CSCs. Thus, tumor cells, especially BCSCs, are able to adapt DNA-damaging anticancer agents (e.g., RT and chemotherapy) via an NF- κ B-controlled prosurvival network, which contributes to the death resistance and aggressiveness of recurrent BC. Therefore, the combination of the intrinsic properties of CSCs and their radiation-adaptive responses due to NF- κ B-regulated genes should be considered targets for the elimination of CSCs and subsequent prevention of tumor recurrence. However, this cannot be achieved until reliable markers for these resistant BCSCs are identified.

BCSCs are recognized by specific biomarkers that are difficult to distinguish from normal somatic stem cells. Creighton *et al.* found that a residual BC cell population that survives after chemo therapy is enriched by cells with both BCSC and mesenchymal features [18]. They defined a gene expression signature common to both CD44⁺/CD24^{-low} and epithelial–mesenchymal transition-associated gene expression. Recently, HER2, a tyrosine kinase receptor involved in BC, has been considered a reliable marker for CSCs [19,20]. *HER2* is a proto-oncogene that encodes a transmembrane protein in various tissues [21]. Probably owing to its high gene dosage, *HER2* overexpression in BC correlates with the aggressiveness and high risk of tumor relapse and is an indicator for poor prognosis [22]. Although no agonist has been identified, the activation of *HER2* is associated with highly invasive tumors. *HER2* activation can be triggered by homo- or hetero-dimerization with other members of the tyrosine kinase receptor family. The development of neutralizing antibodies against the transmembrane domain of the receptor has dramatically improved the outcome of HER2⁺ BC patients identified by FISH and histochemical analyses [23]. The usage of Herceptin®/trastuzumab as an adjuvant treatment sensitizes BC cells to both RT and chemotherapy, and the survival rate is substantially extended for HER2⁺ patients [24]. However, Herceptin is mainly applied to patients with an amplified *HER2* gene [25]. Jones and Buzdar suggest that the efficacy of anti-HER2 therapy is related to the targeting of BCSCs in HER2⁺ patients, which accounts for 28% of BC patients [26]. Supporting this hypothesis is the capacity of *HER2* overexpression to promote the enrichment of stem cells in both normal and malignant cells, even HER2⁻ BC cells [19,20]. The remaining question is whether the enrichment of HER2⁺ BCSCs was related to the death or low expansion rate of HER2⁻ BCSCs, or to the transformation of HER2⁻ CSCs to HER2⁺ cells, especially during the process of chemotherapy and RT. The other key question is whether the role of HER2 is limited only to patients in whom the *HER2* gene is amplified. Guo *et al.* reported that *HER2* gene expression could be induced in response to radiation [27], and Magnifico *et al.* showed that, compared with parental BC cells, *HER2* is highly expressed in CSCs, rendering them sensitive to Herceptin. However, the enhanced HER2 protein level may not be only related to *HER2* gene dosage, but also to the activation of *HER2* gene expression at the transcriptional level via the NF- κ B signaling pathway [28,29]. Furthermore, the HER2 protein level is also linked to post-transcriptional modifications [30]. Therefore, *HER2* expression in BC appears to be a dynamic process and depends on intrinsic (genetic inheritance) and/or extrinsic factors (RT, chemotherapy and cytokines); such changes could

alter the dynamics of the CSC subpopulation pool size during or after anti cancer treatment. Taken together, these data suggest that, besides the gene dosage in a given tumor cell, *HER2* gene transcription and post-translational modification are involved in the adaptive resistance of tumors to RT and RT/chemotherapies. In these cases, NF- κ B-mediated *HER2* transcription may account for the enrichment of the CSC population in the tumors that are resistant to RT and chemotherapy.

Therefore, *HER2* may serve as a biomarker for BCSCs, and signaling pathways involved in its regulation and/or mediating its effects should be taken into account when targeting CSCs [26]. RT has been shown to promote the enrichment of CSCs through the induction of *HER2* expression [19], and this is related to the capacity of radiation-induced NF- κ B to bind and activate the *HER2* gene promoter [28]. However, it has been noticed that *HER2*⁺ BCSCs can be detected not only in *HER2*⁺ tumor cells, but also in *HER2*^{-low} BC [19]. These results highlight an effect of irradiation in the repopulation of BCSCs due to changes in *HER2* expression status. In support of this, Malik *et al.* found that the bone micro-environment affects *HER2* expression in MCF7 cells (*HER2*^{-low}), suggesting that *HER2* status is also related to the host microenvironment tissue, where metastatic tumor growth occur [31]. Thus, due to the critical functions of *HER2* in the promotion of CSCs, the dynamic patterns of *HER2* levels should be taken into account when designing therapeutic protocols, even for BC with *HER2*^{-low} status. Furthermore, the presence of *HER2*⁺ BCSCs in *HER2*^{-low} BC may explain the benefit of anti-*HER2* therapy observed in patients for whom *HER2* gene amplification was not detected. Elucidating the radioresistance mechanisms of BCSCs, such as the *HER2*-STAT3 pathway [19,32], will provide additional new insights into factors involved in the formation of radioresistant BCSCs.

Conclusion

We conclude that BCSCs are responsible, at least in part, for the radioresistant phenotype of recurrent and metastatic lesions of BC. Targeting chemo/radio-resistant BCSCs is a potentially effective approach to obtain total remission and further enhance the cure rate for BC patients. The identification of *HER2*⁺ BCSCs in *HER2*⁻ tumors will enable us to discover new biomarkers in order to trace therapy-resistant BCSCs and to target/kill potential early-stage metastatic lesions. Thus, not only should the *HER2* gene dosage detected in a given tumor biopsy be taken into count when designing a proper treatment plan, but also the dynamic pattern of *HER2* protein expression, as well as *HER2*-associated signaling pathways. In this regard, NF- κ B-mediated *HER2* over expression in tumor cells, progression and repopulation due to the enhancement of radioresistant BCSCs should be investigated further as potential effective targets to treat recurrent BC.

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Biography

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References

1. Lacroix M. Significance, detection and markers of disseminated breast cancer cells. *Endocr. Relat. Cancer*. 2006; 13(4):1033–1067. [PubMed: 17158753]
2. Vreugdenburg TD, Willis CD, Mundy L, et al. A systematic review of elastography, electrical impedance scanning, and digital infrared thermography for breast cancer screening and diagnosis. *Breast Cancer Res. Treat.* 2013; 137(3):665–676. [PubMed: 23288346]
3. Chen RC, Lin NU, Golshan M, et al. Internal mammary nodes in breast cancer: diagnosis and implications for patient management – a systematic review. *J. Clin. Oncol.* 2008; 26(30):4981–4989. [PubMed: 18711171]
4. Liu S, Wicha MS. Targeting breast cancer stem cells. *J. Clin. Oncol.* 2010; 28(25):4006–4012. [PubMed: 20498387]
5. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. *Oncogene*. 2004; 23(43):7274–7282. [PubMed: 15378087]
6. Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat. Rev. Cancer*. 2008; 8(7):545–554. [PubMed: 18511937]
7. Visvader JE. Cells of origin in cancer. *Nature*. 2011; 469(7330):314–322. [PubMed: 21248838]
8. Vlashi E, McBride WH, Pajonk F. Radiation responses of cancer stem cells. *J. Cell Biochem*. 2009; 108(2):339–342. [PubMed: 19623582]
9. Phillips TM, McBride WH, Pajonk F. The response of CD24^{low}/CD44⁺ breast cancer-initiating cells to radiation. *J. Natl Cancer Inst.* 2006; 98(24):1777–1785. [PubMed: 17179479]
10. Effects of radiotherapy and surgery in early breast cancer. An overview of the randomized trials. Early Breast Cancer Trialists' Collaborative Group. *N. Engl. J. Med.* 1995; 333(22):1444–1455. [PubMed: 7477144]
11. Bentzen SM, Agrawal RK, Aird EG, et al. START Trialists' Group. The UK Standardisation of Breast Radiotherapy (START) Trial B of radiotherapy hypofractionation for treatment of early breast cancer: a randomised trial. *Lancet*. 2008; 371(9618):1098–1107. [PubMed: 18355913]
12. Jameel JK, Rao VS, Cawkwell L, et al. Radioresistance in carcinoma of the breast. *Breast*. 2004; 13(6):452–460. [PubMed: 15563851]

13. Ahmed KM, Li JJ. NF-kappa B-mediated adaptive resistance to ionizing radiation. *Free Radic. Biol. Med.* 2008; 44(1):1–13. [PubMed: 17967430]
14. Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nat. Immunol.* 2002; 3(3):221–227. [PubMed: 11875461]
15. Guo G, Yan-Sanders Y, Lyn-Cook BD, et al. Manganese superoxide dismutase-mediated gene expression in radiation-induced adaptive responses. *Mol. Cell. Biol.* 2003; 23(7):2362–2378. [PubMed: 12640121]
16. Ahmed KM, Li JJ. ATM–NF-kappaB connection as a target for tumor radiosensitization. *Curr. Cancer Drug Targets.* 2007; 7(4):335–342. [PubMed: 17979628]
17. Holley AK, Xu Y, St Clair DK, et al. RelB regulates manganese superoxide dismutase gene and resistance to ionizing radiation of prostate cancer cells. *Ann. NY Acad. Sci.* 2010; 1201:129–136. [PubMed: 20649549]
18. Creighton CJ, Li X, Landis M, et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc. Natl Acad. Sci. USA.* 2009; 106(33):13820–13825. [PubMed: 19666588]
19. Duru N, Fan M, Candas D, et al. HER2-associated radioresistance of breast cancer stem cells isolated from HER2-negative breast cancer cells. *Clin. Cancer Res.* 2012; 18(24):6634–6647. [PubMed: 23091114]
20. Korkaya H, Liu S, Wicha MS. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. *J. Clin. Invest.* 2011; 121(10):3804–3809. [PubMed: 21965337]
21. Olayioye MA. Update on HER-2 as a target for cancer therapy: intracellular signaling pathways of ErbB2/HER-2 and family members. *Breast Cancer Res.* 2001; 3(6):385–389. [PubMed: 11737890]
22. Holbro T, Beerli RR, Maurer F, et al. The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proc. Natl Acad. Sci. USA.* 2003; 100(15):8933–8938. [PubMed: 12853564]
23. Chen JS, Lan K, Hung MC. Strategies to target *HER2/neu* overexpression for cancer therapy. *Drug Resist. Updat.* 2003; 6(3):129–136. [PubMed: 12860460]
24. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N. Engl. J. Med.* 2005; 353(16):1659–1672. [PubMed: 16236737]
25. Gonzalez-Angulo AM, Morales-Vasquez F, Hortobagyi GN. Overview of resistance to systemic therapy in patients with breast cancer. *Adv. Exp. Med. Biol.* 2007; 608:1–22. [PubMed: 17993229]
26. Jones KL, Buzdar AU. Evolving novel anti-HER2 strategies. *Lancet Oncol.* 2009; 10(12):1179–1187. [PubMed: 19959074]
27. Guo G, Wang T, Gao Q, et al. Expression of ErbB2 enhances radiation-induced NF-kappaB activation. *Oncogene.* 2004; 23(2):535–545. [PubMed: 14724581]
28. Cao N, Li S, Wang Z, et al. NF-kappaB-mediated HER2 overexpression in radiation-adaptive resistance. *Radiat. Res.* 2009; 171(1):9–21. [PubMed: 19138055]
29. Magnifico A, Albano L, Campaner S, et al. Tumor-initiating cells of HER2-positive carcinoma cell lines express the highest oncoprotein levels and are sensitive to trastuzumab. *Clin. Cancer Res.* 2009; 15(6):2010–2021. [PubMed: 19276287]
30. Feng C, Neumeister V, Ma W, et al. Lin28 regulates HER2 and promotes malignancy through multiple mechanisms. *Cell Cycle.* 2012; 11(13):2486–2494. [PubMed: 22713243]
31. Malik F, Korkaya H, Clouthier SG, et al. Lin28 and HER2: two stem cell regulators conspire to drive aggressive breast cancer. *Cell Cycle.* 2012; 11(15):2780–2781. [PubMed: 22833047]
32. Korkaya H, Kim GI, Davis A, et al. Activation of an IL6 inflammatory loop mediates trastuzumab resistance in HER2⁺ breast cancer by expanding the cancer stem cell population. *Mol. Cell.* 2012; 47(4):570–584. [PubMed: 22819326]

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