ELSEVIER

Seminars in
RADIATION
ONCOLOGY

Breast Cancer Stem Cell Correlates as Predicative Factors for Radiation Therapy

Adam R. Wolfe, BS, and Wendy A. Woodward, MD, PhD

In today's era of personalized medicine, the use of radiation therapy for breast cancer is still tailored to the type of surgery and the stage of the cancer. The future of breast radiation oncology would hopefully entail selecting patients for whom there is a clear benefit for the use of radiation therapy. To get to this point we need reliable predictors of radiation response. Cancer stem cells have been correlated to radiation resistance and outcome for patients with breast cancer, and there is considerable interest in whether cancer stem cell markers or biologic surrogates may be predictive of response to radiation therapy. We review the data or in some cases lack of data regarding stem cell correlates as predictors of radiation resistance as well as the correlation of known predictors with stem cell biology. More research is certainly needed to investigate potential predictors of radiation response, stem cell or otherwise, to move us toward the goal of personalized radiation therapy.
Semin Radiat Oncol 25:251-259 Published by Elsevier Inc.

Introduction

Normal stem cells are defined by 3 characteristics: self-renewal, differentiation into multiple lineages, and long-lived proliferative potential. In the past 15 years, a small population of cells within solid tumors that closely parallels normal stem cells were identified and termed "cancer stem cells" (CSCs).[1] The first studies showed that there are biologically distinct "tumor-initiating" cells of the breast,[2] brain,[3] and hematological system[4,5] that when orthotopically transplanted into immunocompromised mice regenerate solid tumors with neoplastic cells that demonstrate cellular heterogeneity. More recently, several studies of normal mammary gland stem cells have highlighted potential limitations of transplantation and called into question the use of this assay as the gold standard for demonstrating stem cell potential.[6-8] However reviewing the sum of both approaches, Visvader and Stingl[9] suggest that transplantation may unleash a breadth of potential that in vivo is truly seen only at specific moments in development. It is proposed that these cells in cancers mediate recurrence both through their ability to repopulate the tumor with cellular heterogeneity and through innate survival mechanisms to resist therapy. This review focuses on current research on CSCs as predictors of radiation response, specifically in breast cancer, and the reader is directed to the following reviews for a more comprehensive description of mechanisms of radiation resistance in CSCs and CSC treatment strategies.[10,11]

It is important to distinguish between the differences in prognostic and predictive factors because these terms are sometimes used incorrectly in the literature. "A prognostic factor is a clinical or biologic characteristic that is objectively measurable and that provides information on the likely outcome of the cancer disease in an untreated individual. Such prognostic markers are helpful for identifying patients with cancer who are at high risk of metastatic relapse and therefore potential candidates for adjuvant systemic treatments. In contrast, a predictive factor is a clinical or biological characteristic that provides information on the likely benefit from treatment (either in terms of tumor shrinkage or survival). Such predictive factors can be used to identify subpopulations of patients who are most likely to benefit from a given therapy."[12] We examine the data for predictive value of reported putative CSC cells markers (Table 1) in the setting of radiation therapy using breast cancer as a primary focus.

Department of Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.
The authors declare no conflicts of interest.
Address reprint requests to Wendy A. Woodward, MD, PhD, Department of Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030. E-mail: wwoodward@mdanderson.org

http://dx.doi.org/10.1016/j.semradonc.2015.05.009
1053-4296/Published by Elsevier Inc.

Table 1 Studies Correlating Putative Stem Cell Markers to Breast Cancer Outcomes

References	Patient Cohort	Study Size	Hazard Ratio	P	Notes
CD44/CD24					
Giordano et al[21]	Early stage	108	808	0.002	DFS of CSCs in BM
Abraham et al[22]	Mixed	136	NS	NS	DFS and OS
Bernardi et al[23]	Mixed Familial	95	NS	NS	DFS and OS
Bane et al[24]	Breast cancers	364	NS	NS	Associated with poor prognostic features and basal subtype but not predicting OS
ALDH1					
Ginestier et al[33]	Mixed	577	1.76	0.028	Cox multivariate analysis of 5-year OS
Woodward et al[34]	M0	121	4.93	0.04	Independent predictor on multivariable analysis of worse overall survival
Morimoto et al[35]	Mixed	203	1.52	0.459	RFS on multivariate analysis. Trend on univariate analysis $P = 0.56$
Charafe-Jauffret et al[37]	IBC	109	2.7	0.012	Multivariate specific survival
Gong et al[38]	IBC	74	NS	NS	DFS and OS
EZH2					
Reijm et al[42]	ER-positive metastatic	250	1.41	0.017	PFS
Debeb et al[43]	IBC	62	6.5	0.077	LRFS in non-TNBC receiving radiation
26S proteasome					
Langlands et al[47]	Mixed	157	2.9	0.009	LR risk multivariate regression analysis
Cholesterol					
Lacerda et al[50]	Stage III IBC	519	0.4	0.049	Stain use yes vs no, LRR
Wolfe et al[51]	IBC	193	3.21	0.015	HDL $<$ 60 mg/dL 5-year OS

Abbreviations: BM, bone marrow; CSCs, cancer stem cells; DFS, disease-free survival; HDL, high density lipoprotein; LR, local regional; LRFS, locoregional free survival; NS, not specified; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival; TNBC, triple-negative breast cancer.

Putative CSCs Markers

CD44 and CD24

CD44 is a multifunctional Class I transmembrane glycoprotein receptor for hyaluronic acid.[13] It is highly expressed in all cancers and associated with proteins regulating cell adhesion, growth, survival, migration, angiogenesis, and differentiation.[14] CD24 is a small cell surface protein molecule anchored by glycosyl-phosphotidyl-inositol in a wide variety of cancer cells. It is heavily glycosylated and functions in cell-cell and cell-matrix interactions.[15] Functionally, it is identified as an alternate ligand for P-selectin, an adhesion receptor on platelets and endothelial cells.[16] The pioneering study by Al-Hajj et al[2] showed that as few as 100 $CD44^+CD24^{-/low}$ $Lineage^-$ cells in patients with breast cancer could form tumors in mice, whereas tens of thousands of cells with alternative phenotypes failed to form any tumors. It has now been established that $CD44^+/CD24^-$ cells exhibit undifferentiated basal and mesenchymal cell properties and $CD44^+/CD24^+$ cells exhibit highly differentiated basal and epithelial cell properties.[17] Breast cancers resistant to chemotherapy contained higher levels of $CD44^+/CD24^-$ cells.[18] Phillips et al demonstrated that MCF-7 primary mammospheres were more resistant to radiation than cells grown as monolayer cultures and these primary mammospheres had a higher percentage of $CD44^+/CD24^{-/low}$ cancer-initiating cells compared with the monolayer MCF-7 cells. Fractionated doses of irradiation increased activation of Notch-1 and the percentage of the $CD44^+/CD24^{-/low}$ cancer stem or -initiating cells in the nonadherent cell population of MCF-7 monolayer cultures.[19]

Recent studies have been inconclusive regarding the prognostic or predictive value of CD44 and CD24 expression in breast cancer; however, the patient populations studied and the method of study are diverse and likely contribute to variation in results.[20] A definitive, well-designed prospective evaluation to address this question has not been undertaken. In a study by Giordano and colleagues, bone marrow aspirates were collected at the time of surgery from 108 patients with early-stage breast cancer, and CSCs were identified by $CD45^-CD326^+$ expression with a $CD44^+CD24^{-/low}$ phenotype. In multivariate analysis, having CSCs in the bone marrow was an independent predictor for lower disease-free survival (DFS) (hazard ratio = 15.8, $P = 0.017$).[21] Abraham et al investigated breast cancer tissues for the prevalence of $CD44^+CD24^{-/low}$ tumor cells and their prognostic value. The study included paraffin-embedded tissues of 136 patients with and without recurrences. The prevalence of $CD44^+CD24^{-/low}$ tumor cells in 122 tumors was $\leq 10\%$ in most (78%) cases and $> 10\%$ in the other 22%. There was no significant correlation between $CD44^+CD24^{-/low}$ tumor cell prevalence and tumor progression. In this study 70 patients (57%) received radiotherapy and among these patients there was no significant difference in recurrence, event-free survival, or overall survival (OS) in the patients with $< 10\%$ $CD44^+CD24^{-/low}$ compared