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Radiosensitivity and stem cells

Radioresistance of the breast tumor is highly correlated to its level of cancer stem cell and its clinical implication for breast irradiation

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ABSTRACT

Background and purpose: Growing evidence suggested the coexistence of cancer stem cells (CSCs) within solid tumors. We aimed to study radiosensitivity parameters for the CSCs and differentiated tumor cells (TCs) and the correlation of the fractions of CSCs to the overall tumor radioresistance.

Material and methods: Surviving fractions of breast cancer cell lines were analyzed using a dual-compartment Linear-quadratic model with independent fitting parameters: radiosensitive α_{TC}, β_{TC}, α_{CSC}, β_{CSC}, and fraction of CSCs f. The overall tumor radio-resistance, the biological effective doses and tumor control probability were estimated as a function of CSC fraction for different fractionation regimens. The pooled clinical outcome data were fitted to the single- and dual-compartment linear-quadric models.

Results: CSCs were more radioresistant characterized by smaller α compared to TCs: $\alpha_{TC} = 0.1 \pm 0.2$, $\alpha_{CSC} = 0.04 \pm 0.07$ for MCF-7 ($f = 0.1\%$), $\alpha_{TC} = 0.08 \pm 0.25$, $\alpha_{CSC} = 0.04 \pm 0.18$ for SUM159PT ($f = 2.46\%$). Higher f values were correlated with increasing radioresistance in cell lines. Analysis of clinical outcome data is in accordance of a dual-compartment CSC model prediction. Higher percentage of BCSCs resulted in more overall tumor radioresistance and less biological effectiveness.

Conclusions: Percentage of CSCs strongly correlated to overall tumor radioresistance. This observation suggested potential individualized radiotherapy to account for heterogeneous population of CSCs and their distinct radiosensitivity for breast cancer.

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Radiotherapy has been widely used for cancer treatment either by itself or in conjunction with other treatment types such as surgery and chemotherapy [1]. Despite advanced delivery approaches, improved radiation delivery precision and aggressive fractionation schemes, tumor progression and recurrence are frequently observed [2–9]. The Metastatic Breast Cancer Network showed that 20–30% of people initially diagnosed with early stage disease would develop metastatic breast cancer [10].

Recent cancer cell research and clinical data suggest that successful cancer therapy must eradicate the cancer stem cells (CSCs) [11–18]. Current stem cell theory supports the following highlighted characteristics of CSCs: (1) Solid tumors, such as breast, brain, prostate, lung comprise a distinct heterogeneous population of CSCs; (2) CSCs are tumorigenic and markedly different than the differentiated cancer cells in their ability to proliferate and self-renew [11,12]; (3) CSCs are more persistent in tumors and cause relapse and metastasis by giving rise to new tumors; (4) CSCs can be found at any location in the tumor [16–18] and (5) are generally more resistant to ionization radiation or other cell killing agents [14,20]. The presence of CSC challenges the limitations of current treatment method and suggests a significant opportunity to design more effective cancer treatments. CSC-targeted approaches include sensitization of CSCs to conventional drugs, promoting CSC differentiation, targeting and blocking relevant CSC signaling pathways and destroying CSC niches [22].

It is well known that individual patients respond differently when subject to the same radiotherapy regimen. The differences have been attributed to patient-specific tumor and normal tissue radiosensitivity and patient characteristics including age, performance status, staging, disease sites and metastases location. Intrinsic tumor radiobiology not only differs for different types of cancer, it also varies for the same cancer in different patients. Biologically guided personalized radiotherapy based on molecular prognostic and predictive biomarkers is expected to profoundly impact radiation therapy [1]. Due to the markedly different CSC radiobiology and their varying presence in tumors, radiotherapy may be

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individualized accordingly. The aims of this study were twofold: (1) to investigate tumor radioresistance and its correlation to the level of CSCs through *in vitro* experiments, developing a two-compartment mathematical model determining radiosensitivity of both BCCs and BCSCs; (2) to validate the two-compartment CSC model using pooled clinical outcome data. The potential clinical implication suggested that personalized therapy based on pre-clinical biological characteristics, e.g., the fractions of cancer stem cells, could lead to improved clinical outcomes.

Method and materials

Surviving fraction from in vitro experiment

In vitro surviving fractions for four established breast cancer cell lines, including MCF-7, T47D (Luminal A), MDA-MB-231 (claudin-low), SUM159PT (triple-negative) molecular phenotypes, were digitized from Lagadec et al. [20]. These assays were irradiated using single doses of 0, 2, 4, 6, 8 or 10 Gy under acute hypoxic conditions (2 h, 0.1% O_2) or normoxia (atmospheric conditions, 21% O_2). In this study, we re-analyzed the survival rate for the four cell lines at atmospheric conditions. The factions of BCSCs in each cell line were identified using two markers as described in [19–21].

Dual-compartment survival fraction model

The linear-quadratic (LQ) model [23–25] is the most commonly used radiation-induced cell-killing model. To account for the presence of the breast CSCs in addition to the tumor cells (TCs), a more generalized dual-compartment survival fraction model was introduced:

$$S = f' \times e^{(-\alpha_{TC}D-\beta_{TC}D^2)} + (1-f') \times e^{(-\alpha_{CSC}D-\beta_{CSC}D^2)} \quad (1)$$

where S is the total cell surviving fraction, α_{TC}, β_{TC} and α_{CSC}, β_{CSC} characterize intrinsic radiosensitivity of the tumor cells and CSCs respectively, and D is the radiation dose. The α/β ratio measures the relative importance of the linear and quadratic terms in the LQ model. The fraction of TCs to the total plated cells within a certain cell line (or a given tumor) is given by f', which was determined by the aforementioned CSC assays. When $f' = 1$ (no CSC), the dual-compartment model became the single-compartment tumor cell surviving model (LQ model). The least square method was used to determine the model parameters by fitting the cell surviving fractions as a function of radiation dose. The best-fit curve is defined as the parameter set that has the minimal sum of the deviations squared calculated by Eq. (2):

$$\chi^2 = \sum_{j=1}^{n} \frac{[S^{Calc}(D_j) - S^{Meas}(D_j)]}{\sigma_j^2} \quad (2)$$

where, $S^{Calc}(D_j)$ is the j-th survival rate calculated from Eq. (1), $S^{Meas}(D_j)$ is the observed survival rate for the given dose D_j; σ_j^2 is the error for the j-th data point.

The Function Minimization and Error Analysis package (MINUIT) from the European Laboratory for Participle Physics program library (CERNLIB, Geneva, Switzerland) was employed to fit the data (http://cernlib.web.cern.ch/cernlib).

Overall tumor radiosensitivity

The overall tumor radiosensitivity (α) was estimated by the radiosensitivities of the TCs, CSCs and their fraction by number in a heterogeneous tumor:

$$\alpha = \alpha_{TC} \times f' + \alpha_{CSC} \times (1-f') \quad (3)$$

where f', α_{TC} and α_{CSC} were defined aforementioned.

Analysis of clinical outcome data

The generalized LQ model [23–25] is widely adopted in radiation oncology for assessing tumor control and normal tissue complications. The reported clinical local disease-free survival rate (LSR) at arbitrary time points of 5- and 8-years was assessed using a Poisson model.

$$LSR(D) = e^{-K \cdot S} \quad (4)$$

where K is the number of clonogenic cells, and S is the cell surviving fraction.

To avoid overfitting, the number of variables was reduced. We adopted the plausible parameter set for TCs [26], the parameters associated with BCSCs including f, α_{CSC}, Td (CSC) were determined by the data-fitting program. As a simple approximation, we assumed $\beta_{CSC} = 0$, which was widely observed and used in many recent publications [11,12].

Pooled clinical data

The dual-compartment model was validated using a pooled clinical dataset of breast cancer patients [2–6]. Whelan et al. [2] compared short term (42.5 Gy/16 fractions over 22 days) and long-term (50 Gy/25 fractions over 35 days) fractionation schedules based on a randomized trial of 1234 patients. Owen et al. [3] studied the effectiveness of fractionation schemes: 50 Gy (25 fractions), 39 Gy (13 fractions) and 42.9 Gy (13 fractions) for a total of 1410 women with early-stage breast cancer at long-term follow-up. Two large randomized trials in the United Kingdom Standardization of Breast Radiotherapy (UK START) trial A (2236 patients) and trial B (2215 patients) [4] and [5] were also included. START A included randomized comparisons of 41.6 Gy (13 fractions) and 39.0 Gy (13 fractions) over 5 weeks; START B compared the local–regional tumor relapse rates and late adverse effects for patients received 40 Gy (15 fractions) over 3 weeks, with a control schedule of 50 Gy (25 fractions) over 5 weeks.

Results

Fig. 1 shows surviving fractions for breast cancer cells in clonogenic and mammosphere assays under atmospheric conditions. f is the percent of BCSCs in each cell line. The symbols (experimental data) were fitted using the dual-compartment model (curves). Fig. 1 (a) surviving fractions of MCF-7 ($f = 0.1\%$) and SUM159PT ($f = 2.46\%$) cell lines in clonogenic assay; (b) surviving fractions of MCF-7 ($f = 0.98\%$) and SUM159T ($f = 8.68\%$) cell lines in mammosphere assays; (c) surviving fractions of T47D cell line in clonogenic survival assays (0.9%) versus mammosphere assays ($f = 1.34\%$) and (d) surviving fractions of MDA-MB-231 in clonogenic survival assay ($f = 1.18\%$) versus mammosphere assays ($f = 2.04\%$), respectively. In Fig. 1 (d), the cell surviving curve from 0 to 4 Gy describe mostly cell killing while the curve from 4 Gy or higher dose range is composed of cell killing and induction of tumorigenic/spherogenic cells, which was not accounted in the two-compartment model.

Higher percentages of CSCs in the cell lines were associated with more radioresistant phenotypes characterized by less curvy lines. The fractions of CSCs in mammosphere assays were greater than corresponding cell lines. Correspondingly, there was almost no shoulder found in the surviving curves (more radioresistant in mammosphere assays) with β_{CSC} approximately zero.

Fig. 2 illustrates the derived radiosensitive parameters *of* α_{TC} and α_{CSC} as a function of percentage of CSCs in (a) clonogenic assays and (b) mammosphere assays, respectively. The derived α_{CSC} was found smaller than α_{TC} with $p = 0.02$, $p = 0.003$ for clonogeneic and mammosphere assays respectively, indicating that the BCSCs