Optical Scattering as an Early Marker of Apoptosis during Chemotherapy and Antiangiogenic Therapy in Murine Models of Prostate and Breast Cancer

Syeda Tabassum,1 Anup Tank,2 Fay Wang,2 Kavon Karrohi,2 Cameron Vergato,3 Irving J. Bigio,1,2 David J. Waxman,2 and Darren Roblyer1,2,*
1Electrical & Computer Engineering, Boston University, 8 Saint Mary’s Street, Boston, MA 02215, USA
2Biomedical Engineering, Boston University, 44 Cumming Mall, Boston, MA 02215, USA
3Division of Cell and Molecular Biology, Department of Biology and Bioinformatics Program, 5 Cumming Mall, Boston, MA 02215, USA

Abstract: Optical scattering parameters were correlated with markers of apoptosis and proliferation in preclinical tumor models, and outperformed tumor volume and functional parameters in treatment response prediction.

1. Introduction

Surveillance of the in vivo tumor state during the course of drug treatment is essential for understanding the efficacy of anti-cancer agents in the preclinical setting [1]. Here we focus on optical scattering, known to be sensitive to sensitive to tissue micro-architectural changes, as a novel marker of anti-cancer therapy response [2]. There has not been, until recently, a method to quantify optical scattering over a wide-field, such as over the tumor surface in preclinical tumor models. Here, we exploit spatial frequency domain imaging (SFDI), as a new wide-field and label-free imaging modality to quantify the reduced optical scattering coefficient (μ′s) and the absorption coefficient (μa) [3]. SFDI belongs to a larger class of diffuse optical imaging (DOI) techniques, many of which have been translated to the clinic for diagnostic and prognostic applications in breast, prostate, and other solid tumors [4]. We set two goals for the present work: 1) to determine the ability of μ′s to predict early treatment response in clinically-relevant tumor models; and 2) to investigate the biological correlates of μ′s and other SFDI metrics in drug-treated versus control tumors. To accomplish these goals, we first tracked μ′s-based parameters in prostate and breast xenograft models following treatment. We then correlated SFDI metrics with tumor physiological markers in a cross-sectional animal study conducted over multiple weeks. Finally, we compared the utility of optical scattering as an early marker of treatment response against other commonly utilized parameters, including anatomic tumor volume and functional measurements (e.g. tumor oxygen saturation) [5].

2. Materials and Methods

Detailed descriptions of SFDI instrumentation and data analysis are provided elsewhere [3]. Briefly, SFDI utilizes projections of spatially modulated visible and/or NIR light to extract intrinsic tissue optical properties (μ′s, μa). A power law was used to fit the wavelength dependence on μ′s to derive the scattering amplitude (a) and scattering power (b). The measured values of μa were used to extract tissue chromophore concentrations using Beer’s Law which yields tissue-level concentrations of oxyhemoglobin (ctHbO2) and deoxyhemoglobin (ctHb) [3]. From these, additional hemodynamic parameters were indirectly derived, including total hemoglobin content (ctTHb = ctHbO2 + ctHb) and oxygen saturation (StO2 = (ctHbO2/ctTHb) × 100).

Severe combined immunodeficient (SCID) hairless outbred mice (SHO-PrkdcscidHhr; Charles River Laboratories) bearing PC3/2G7 tumors were randomly assigned to treatment groups when the average group tumor volume (TV) reached ~500 mm3: control (drug-free) group, N=33 mice; cyclophosphamide (CPA) treatment, N=25 mice; DC101 treatment, N=29 mice. CPA monohydrate was administered on a metronomic schedule every 3 days for 3 cycles. DC101 was given every 3 days for 6 cycles. A separate study was conducted with E0771 mammary tumor model to contrast observed trends in optical changes against a second tumor type. Mice were imaged longitudinally with SFDI. Several SCID mice from each group were euthanized for tissue analysis on days 0, 1, 9, 18, and 26 (at least N=4 per group on each day). Tumors were frozen or fixed for cross-sectional analysis by Immunohistochemistry (IHC) to investigate apoptosis (cleaved caspase-3), cell proliferation (PCNA), blood vessel density (CD31), glucose uptake (Glut-1), macrophage infiltration (Mac-1), and vessel patency (leakiness: Hoechst assay). Imaging and treatment details on C57BL/6 mice bearing E0771 tumors are provided elsewhere [5].

3. Results

3.1 Optical scattering parameters correlate with apoptosis and proliferation

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Here, we summarize our hypotheses for potential SFDI and IHC correlations and references to previous literature supporting the proposed correlations [5]. For example, the a parameter decreased in the control group but increased significantly in the treated groups, with both CPA and DC101, during treatment and rebound (Fig. 1C). The opposite was observed for b parameter longitudinally (Fig. 1F). The changes in a were broadly correlated with changes in cleaved caspase-3 (Fig. 1D). The changes in b were likewise correlated with the PCNA trends (Fig. 1G). The Pearson correlations were strong for a versus cleaved caspase-3 (Fig. 1E, Pearson correlation coefficient $\rho_p = 0.75$) and b versus PCNA (Fig. 1H, $\rho_p = 0.69$). The CD31 correlated strongly with ctHbO$_2$ ($\rho_p = 0.85$), ctTHb ($\rho_p = 0.71$), and StO$_2$ ($\rho_p = 0.92$); and the Hoechst assay correlated well with ctHbO$_2$ ($\rho_p = 0.54$), ctTHb ($\rho_p = 0.54$), and StO$_2$ ($\rho_p = 0.52$). Similar trends in a parameter were also observed in CPA-treated E0771 tumors.

3.2 Scattering parameters provide a superior and biologically-specific early response prediction compared to tumor volume and hemodynamic parameters

Discriminant analysis was conducted to determine the accuracy of classifying treated versus control tumors using SFDI parameters as either stand-alone imaging markers, or as companion markers to TV. Tumor volume is used here to contextualize the predictive value of SFDI parameters. A summary of classifier performance is shown in Fig. 2. For CPA, the a parameter yielded better performance than TV on days 1 and 4. When a was combined with TV, superior performance was found on days 3 and 4 as compared to either parameter alone. For DC101, the exponent b provided the best performance on day 1, and the combination of a and TV had superior performance over either single feature on days 3, 4, 6, 7, and 9. At very early time points (i.e., days 1, 3 and 4) the features a and b outperformed TV. Figure 2C,D shows prediction advantage, where a positive predictive advantage indicates that the SFDI feature precedes TV in achieving the same AUC by the indicated number of days, and vice versa. For CPA, within just one day of treatment (1.4), the a parameter provides a predictive power that is not matched by TV until after day 2 (Fig. 2C). For DC101, the b parameter yielded positive values as large as 2 days until day 4 (Fig. 2D). The a parameter for DC101 preceded TV starting at day 4.
Optical scattering centers

Figure 2. A,B. Discriminant classification analysis for discrimination of treated versus control tumors. AUC values of well-performing stand-alone features are shown in A for CPA and in B for DC101. C,D. Predictive advantage (in days) for the a or b parameter compared to TV.

4. Discussion and Conclusion

The a and b optical scattering parameters correlated strongly with markers for apoptosis and cell proliferation, respectively. We hypothesize that as cells break down during apoptosis, the density of optical scattering centers increases due to morphological changes including chromatin condensation and mitochondrial changes, thereby increasing the a parameter. Conversely, during proliferation, an increase in cell density may alter the distribution of scattering particle sizes, leading to a higher proportion of smaller scattering centers, which causes b to increase. Both parameters provided high imaging contrast between treated and control tumors, starting at early time points.

The classification analysis revealed the scattering a parameter as the best performing single SFDI feature for CPA, and a + TV as the best performing dual feature during either treatment. Regression analysis demonstrated that the a parameter has predictive advantage over TV in CPA, whereas the b parameter exhibited a better prediction advantage than TV at the beginning of DC101 treatment. We speculate that these differences may reflect the different biological mechanisms of these drugs. CPA is an alkylating agent that can induce tumor apoptosis within hours of treatment, plausibly supporting the parameter. Conversely, DC101 targets vascular endothelial growth factor receptor 2 causing vessel regression which conceivably inhibits proliferation before inducing apoptosis at later time points, caused by oxygen and/or nutrient starvation, which may explain the early predictive power of the b parameter, followed later by the predictive power of the a. We also note that both a and b changed dramatically over time in treated tumors, even though treatment resulted in tumor stasis with little to no change in TV during the treatment period. Thus, the a and b parameters may provide unique insight during monitoring of treatment response, which could supplement measurements of tumor size. Optical scattering remains largely unexplored in the context of treatment monitoring, and our findings suggest that optical scattering should be further investigated with clinical DOI tools. Going forward, widefield measurements of quantitative optical scattering measured with SFDI may represent a powerful new contrast for exploring new drugs and therapeutic strategies in the preclinical setting.

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