Title: Enhancement of Hemodynamic Contrast in the Cancerous Breast by Carbogen Inspiration

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Hallmarks of the tumor phenotype include increased stiffness [1], enhanced angiogenesis with sluggish perfusion [2], increased vascular leakiness leading to increased interstitial pressures [3], and increased metabolic demand [2]. Separately, it is known that the vascular autoregulation mechanism normally achieves a tight coupling between the vascular supply and prevailing metabolic demand, but that the fidelity of vascular autoregulation may be attenuated in tumor tissue as a consequence of alterations in the vascular endothelium and surrounding vascular smooth muscle [2]. One consequence of this is that many tumor types operate on the brink of hypoxemia, suggesting that the otherwise enhanced supply actually is limited, perhaps as a consequence of disturbances in hydrostatic pressures caused by vascular leakiness and changes to the interstitium scaffolding [1].

Based on the preceding considerations, we hypothesized that manipulations of the oxygen supply-demand balance may produce responses that differ markedly between the tumor and surrounding healthy tissue. One approach that has recently been explored is the inspiration of carbogen [4]. While the carbogen response is tissue-specific, the most commonly seen response is vasodilation as a consequence of the effects of elevated CO_2 (elevated oxygen levels *per se* typically cause vasoconstriction) [4]. Here we explore the response of the healthy and tumor-bearing breast to a carbogen mixture consisting of 98% O_2 and 2% CO_2, as a basis for producing additional modulation on the oxygen supply-demand balance.

Simultaneous bilateral breast imaging was performed using an imaging system recently described by Al abdi *et al*. [5], which provides for high optode-density dynamic fNIRS imaging, at rest and in response to physiological manipulations. Collection of measurements from both breasts simultaneously allows for subsequent use of paired difference analyses that cannot be considered using other fNIRS breast imaging systems. After giving informed consent and providing a brief medical history, research participants were seated and the sensing heads were adjusted to make good contact with both breasts. Following a five-minute baseline scan while room air was breathed, a facemask was applied and subjects breathed the carbogen mixture for an additional five minutes. Optical data were analyzed offline: application of a low-pass filter with a 0.2-Hz cutoff frequency was followed by use of the Normalized Difference Method to reconstruct tissue oxygen saturation (HbSat) and blood volume (HbT) images [6].

Imaging results obtained from 48 subjects (16 breast cancer, 18 benign pathology, 14 healthy control) are consistent with the hypothesis that carbogen and room air have different impact on microvasculature in healthy and tumor tissue, comparable to a previously reported effect seen when comparing pure oxygen and carbogen [4]. In particular, we have found that tumor tissue shows a larger drop in HbT and concomitant rise in HbSat in comparison to healthy tissue. Also noteworthy is the finding that image contrast is substantially improved by transforming the (HbSat,HbT) image values to a measure of the statistical extremeness (i.e., the Mahalanobis distance [7]) for each pixel. Furthermore, the preceding effect is enhanced by referencing the image-pixel data of one breast to the distribution of image values for the contralateral breast. This is a concrete demonstration of the above-mentioned utility of the simultaneous dual-breast measurement approach. Thus we have demonstrated that controlled manipulation of the tissue oxygen supply-demand balance can enhance the detectability of breast cancer by exploiting known physiological abnormalities of tumor tissue.

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