Quantification of Necrosis in Animal Tumor Model using K-Means Clustering of ADC

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Introduction

Diffusion-weighted (DW) MR imaging has been shown to be sensitive to tumor cellularity and necrosis [1]. Cancer imaging studies often require estimates of tumor necrotic fraction and segmentation of necrotic areas, which can be done by applying an ADC threshold or using a variety of clustering methods [2,3]. We explored quantification of tumor necrosis based on k-means clustering of ADC data in a xenograft model.

Methods

Six female athymic nu/nu rats (6–8 wks old; weight, 200–280 g) with HT29 human colorectal tumors (volume ~700 mm^3) grown subcutaneously in right hind limb were imaged as a control group in a drug response study. Rats were imaged twice, at baseline and after receiving three doses of vehicle solution (0.5% methylcellulose) by oral gavage over a 48 h period. MRI was performed at 7 T (Bruker BioSpin, Billerica, MA) with a home-built 4 cm diameter Helmholtz coil. Tumors were localized on T2-weighted images. DW images were then acquired in the same location in the center of the tumor with turbo spin echo sequence (TR/TE = 2000/34 ms; 4 averages; FOV = 30x30 mm^2; slice thickness, 0.8 mm; matrix, 128x128; 4 central slices; b = 0, 300, 400, 500, 700, 900 s/mm^2). After imaging, rats were sacrificed, tumors were excised and histologically analyzed. Necrotic areas were outlined and measured on hematoxylin and eosin (H&E) stained sections and necrotic fractions were determined relative to the whole tumor areas. Image analysis was performed in Matlab (Mathworks, Natick, NJ). ADC maps were calculated by fitting signal in every voxel with monoexponential expression, $S(b) = S_0 \exp(-ADC \cdot b)$. Tumors were segmented from the background and k-means clustering was applied to the post-treatment ADC data pooled from all tumors. Clustering was implemented using Matlab kmeans function with k = 2 (cluster 1, viable; cluster 2, necrotic) and k = 3 (cluster 1, viable; cluster 2, mixed; cluster 3, necrotic).

Results

Treatment with vehicle solution did not affect the mean tumor ADC (mean±st dev, $ADC_{pre} = (0.78 \pm 0.25) \times 10^{-3}$ mm^2/s; ADCpost = $(0.74 \pm 0.18) \times 10^{-3}$ mm^2/s, p = 0.6). Distributions of voxel ADC values before and after treatment were nearly identical. The histological necrotic fraction ranged from 0.14 to 0.36. The spatial correspondence between the cluster maps and necrotic areas on histological images was better in tumors with larger contiguous necrotic areas than in tumors with multiple small regions of necrosis (Fig. 1). Clustering post-treatment ADC data with k = 2 yielded the cutoff value $ADC_c = 0.88 \times 10^{-3}$ mm^2/s (Table, Fig. 2a). Similar results were obtained for pre-treatment ADC data. The fraction of cluster 2 correlated well with the histological necrotic fraction (R = 0.95, p = 0.003; Fig. 2b), although in 3/6 tumors necrosis was slightly overestimated. Clustering with k = 3 produced a total sum of distances that was 1.8 times lower than obtained with k = 2. When cluster 3 was considered as necrotic, its fraction was considerably lower than the histological necrotic fraction, although the correlation between the fraction of cluster 3 and histological necrotic fraction was only slightly lower than for k = 2. When clusters 2 and 3 were considered necrotic, their fraction produced a worse estimate of necrosis than the two previous options (k = 2 or k = 3 (cluster 3)).

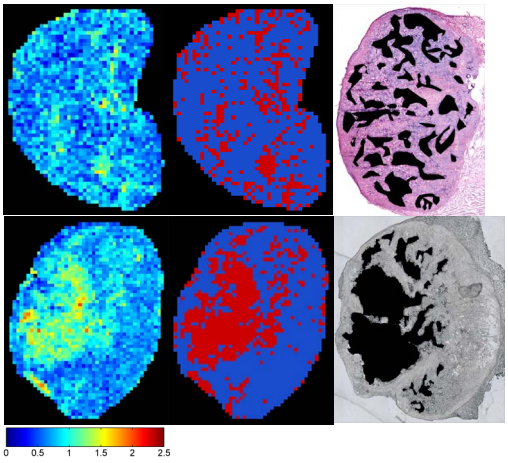

Figure 1: Top row: Tumor with multiple small necrotic regions. Bottom row: Tumor with a large necrotic region. Left: ADC map; middle: k = 2 cluster map (cluster 1: viable (blue), cluster 2: necrotic (red)); right: histology image (H&E stain) from a matching location, with necrotic areas masked.

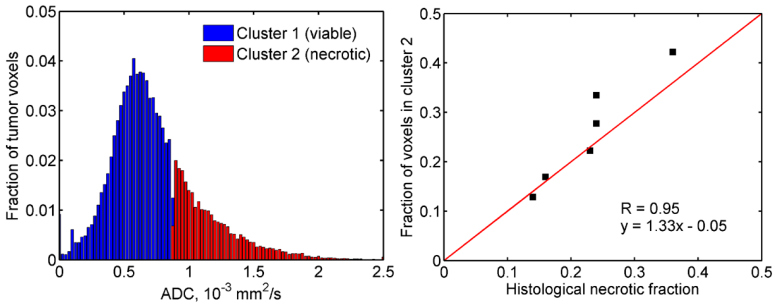

Figure 2: a) Histogram of pooled post-treatment ADC data for k = 2: cluster 1, viable tissue (blue) and cluster 2, necrotic tissue (red) with the cutoff at $ADC_c = 0.88 \times 10^{-3}$ mm^2/s.
b) For k = 2, the fraction of tumor voxels in cluster 2 correlates well with the histological necrotic fraction. The red line is the identity line.

Table: Parameters of k-means clustering of ADC

Number of clusters (necrotic cluster)	Cluster centroids (10^{-3} mm^2/s)	Correlation with histology R (p)	Linear regression with histological necrotic fraction (x)
k = 2 (2)	0.57, 1.2	0.95 (0.003)	1.33x – 0.05
k = 3 (3)	0.45, 0.82, 1.4	0.93 (0.008)	0.85x – 0.07
k = 3 (2 & 3)	0.45, 0.82, 1.4	0.83 (0.04)	1.31x + 0.24

Discussion

Segmentation of ADC using two clusters provides the best correlation and agreement with the histological necrotic fraction. Clustering with k = 3 provided a slightly lower correlation and a considerable underestimation of necrosis. These findings are in agreement with the results obtained by Henning et al. [4] in RIF-1 tumors with similar necrotic fractions (k = 2, R = 0.76; k = 3, R = 0.72). The cutoff between the clusters (0.88×10^{-3} mm^2/s) is close to the empirically determined threshold for ADC of necrotic areas in previous reports [5]. Larger necrotic areas were better identified on ADC maps and cluster maps than smaller islands of necrosis [1] and therefore the accuracy of automatic segmentation of necrotic areas may vary across tumor lines and depend on image quality. Rigorous validation of clustering-based segmentation requires coregistration of DW images or ADC with histopathological sections.

References

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