DCE-MRI-based Tumor Subregion Partitioning with Texture Feature Extraction for Prediction of Ki-67 Status of Estrogen Receptor-Positive Breast Cancers

Ming Fan[1], PhD, Hu Cheng[1], BS, Peng Zhang[1], BS, Xin Gao[2], PhD, Juan Zhang[3], MD, Guoliang Shao[3], PhD, Lihua Li[1], PhD

[1]Institute of Biomedical Engineering and Instrumentation, Hangzhou Dianzi University, Hangzhou, China

[2]King Abdullah University of Science and Technology (KAUST), Computational Bioscience Research Center (CBRC), Computer, Electrical and Mathematical Sciences and Engineering Division (CEMSE), Thuwal, 23955-6900, Saudi Arabia.

[3]Zhejiang Cancer Hospital, Zhejiang Hangzhou, China

Corresponding Author Info

Lihua Li,

College of life information and instrument engineering, Hangzhou Dianzi University, Hangzhou, China, 310018

Phone: +86-571-86878587

Fax: +86-571-87713528

Email: lilh@hdu.edu.cn

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Running Title: Tumor Subregion Partitioning on DCE-MRI

Abstract

BACKGROUND:

The correlation between dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in tumor subregion and Ki-67 status for breast cancer has not been well studied.

PURPOSE:

To predict the Ki-67 status of estrogen receptor (ER)-positive breast cancer patients with an analysis of tumor heterogeneity with subgroup identification based on patterns DCE-MRI.

STUDY TYPE:

Retrospective study.

POPULATION:

There were 77 breast cancer patients with ER-positive breast cancer were investigated in which 51 were low Ki-67 expression.

FIELD STRENGTH/SEQUENCE:

T1-weighted 3.0 T DCE-MR images.

ASSESSMENT:

Each tumor was partitioned into multiple subregions using three methods based on patterns of dynamic enhancement: 1) time to peak (TTP) value, 2) peak enhancement rate (PER), and 3) kinetic pattern clustering (KPC). In each tumor subregion, 18 texture features were computed. The partitioning results were compared to the same feature extraction methods across the whole tumor.

STATISTICAL TESTING:

Univariate and multivariate logistic regression analyses using a leave-one-out-based cross-validation (LOOCV) method.

RESULTS:

In the univariate analysis, the best-performance individual feature was given by texture statistic of sum variance at the postcontrast MR image in the tumor subregion with early TTP for differentiating between patients with high and low Ki-67 expression (Area under the receiver operating characteristic curves, AUC = 0.748). The multivariate analysis showed that texture features from the tumor subregion associated with early TTP yielded highest performance among the subregions with an AUC of 0.807 for predicting the Ki-67 status. Among all regions, the tumor area with high PER at precontrast MR image achieved an AUC of 0.722, while the subregion that exhibited a high overall enhancement rate based on KPC had an AUC of 0.731. The three classifiers based on subregion partitioning methods with texture features significantly ($p<0.01$) outperformed the classifier using texture features from the whole tumor, which produced an AUC of 0.59.

DATA CONCLUSION:

Texture analysis of intratumor heterogeneity has the potential to generate a valuable clinical marker to enhance the prediction of breast cancer prognostic indicators.

Key Words: DCE-MRI; Breast Cancer; Ki-67; Tumor partitioning

INTRODUCTION

Breast tumors can be categorized into molecular subtypes, which include luminal A, luminal B, HER2-positive and basal-like. Estrogen receptor (ER)-positive accounts for approximately 70% of human breast cancer tumors (1), which generally have a favorable prognosis, while a subset of them will experience disease recurrence. Recent studies suggest that ER-positive breast cancer patients with a lower Ki-67 level (less than 14%) more often achieve a pathological complete response (pCR) (2-4), while those with a high Ki-67 level are more likely to show a poor prognosis (5, 6) and are associated with worse survival outcomes in early breast cancer (7). The Ki-67 index has also been a proliferation marker used to distinguish between the luminal A and luminal B molecular subtypes of the ER-positive breast cancer (8). Moreover, this prognostic indicator reflects the extent of proliferative activity, an indicator of tumor aggressiveness (9), and is a reliable identifier of more aggressive growth of breast cancer (5). Therefore, accurate identification of Ki-67 status is of vital importance for prognostic analysis of breast cancer.

Heterogeneity is a common finding in many tumors, including breast cancer (10-13). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is employed as a methodology to evaluate the extent of tumor angiogenesis and tumor heterogeneity by analyzing the patterns of enhancement (14, 15). Many studies have explored heterogeneous enhancement patterns in DCE-MR images within the entire breast tumor to build predictive models of tumor subtypes based on the quantitative evaluation of contrast enhancement (16-21). Shin et al. identified DCE-MRI kinetic parameters

associated with the Ki-67 proliferation status in patients with ER-positive breast cancer (22). A recent study conducted quantitative heterogeneity analyses, such as first-order statistics, shape and morphology, texture and geometry, indicating candidate MR imaging biomarkers for classifying HER2 status (23). Related studies have utilized gray-scale correlation matrix (GLCM) textures of DCE-MRI, which exhibited correlations with the high and low OncotypeDX risk categories for ER-positive cancers (18, 24).

The above analyses of enhancement patterns in DCE-MRI have provided useful information for measurements of the extent of heterogeneity in the entire tumor. However, studies suggest that intratumoral regions display distinct dynamic patterns, which reflect differing fundamental biological processes and differing prognostic potentials (12), and could also possess valuable information that is not effectively captured by radiomic analysis of the entire tumor (25-27). Present studies of intratumor identification mainly focus on discriminating between patients who are responsive or non-responsive to pathological neoadjuvant chemotherapy (27-29). Few studies have examined radiomic features inside tumors for molecular subtype classification (26, 30). Whether a regional analysis within the tumor could be more informative than an analysis of the entire tumor remains unclear and requires more research.

The purpose of this work is to predict Ki-67 proliferation status based on quantitative image features extracted from tumor subregions related to various dynamic enhancement patterns in DCE-MRI.

MATERIALS AND METHODS

Patient cohort

The image data collection protocol was approved by the institutional review board (IRB) with all patients' information removed. Since this was a retrospective study in nature, the informed research consent was waived by IRB. We initially assembled an image dataset of 148 patients between January 2013 and May 2015. All patients had biopsy-proven breast cancer and underwent breast MRI for preoperative staging. Patients without pathologic examination or incomplete pathological data (n=23) were excluded from the dataset. Twenty patients who received radiotherapy or chemotherapy before MR examination were also eliminated from the dataset. In addition, 28 patients with ER-negative results were excluded. The final dataset included 77 ER-positive breast cancer patients for analysis.

Pathologic assessment

The expression of the ER, PR, HER2, and Ki-67 status of each patient with invasive breast cancer was determined using streptavidin-peroxidase (SP) immunohistochemistry (IHC). A sample was scored as ER- and/or PR-positive when at least 1% of the tumor cell nuclei showed staining for ER or PR (31). A sample was considered positive if the Ki-67 level was greater than 14% and was considered negative otherwise.

MR Image acquisition

DCE-MR imaging was performed at 3.0 T using a Siemens Magnetom Verio 3.0 T MRI scanner (Siemens_Medical Solutions, Erlangen, Germany). All patients were scanned in the prone position with a dedicated eight-channel double-breast coil (Siemens

Medical Systems). In each MRI examination, the precontrast series of fat-saturated T2-weighted 3D images were first scanned and acquired, followed by a fat-suppressed T1-weighted imaging. After the intravenous injection of a contrast agent at 2 mL/sec with a dose of 0.2 mmol per kilogram body weight, five postcontrast scans were acquired. The first postcontrast sequence was acquired at 60 seconds after contrast agent injection and the five postcontrast series are sequentially performed in the time interval of 30 seconds. The parameters were as follows: repetition time (TR) = 4.5 msec, echo time (TE) =1.6 msec, flip angle = 10°, field of view (FOV) = 340 × 340 mm, parallel imaging factor =2, and slice thickness = 2.4 mm. The acquisition matrix was 896 × 896 with a spatial resolution of 0.38 × 0.38 × 2.4 mm.

Image preprocessing

The location of the center of the suspicious breast tumor was first annotated in each case retrospectively in consensus by two radiologists (G.S and J.Z) with more than 10 years of experience. Image segmentation was performed at the third series of postcontrast MR images. An initial "seed" was chosen using the labeled tumor's center location. After that, a volumetric breast tumor boundary contour was automatically segmented by a spatial fuzzy C-means (FCM) algorithm and was then refined by a Markov random field (MRF)-based approach, the parameters of which were adaptively adjusted using segmentation results of contiguous slices (32). The segmentation results were further examined by manual correction performed by our investigators, which occurred in less than 10% of all the cases. Regarding cases with multicentric or multifocal tumors, the largest tumor was chosen for analysis. The segmented breast

areas were registered between sequential DCE-MRI images scan series following the same procedure as our previous work (32).

Intratumor partitioning to identify tumor subregions

To examine the kinetic heterogeneity of a tumor, we grouped tumor voxels into homogeneous clusters according to their kinetic patterns for all slices and looked for feature statistics within every cluster. To comprehensively characterize intratumor spatial heterogeneity, we performed tumor partitioning based on the values of TTP, PER and KPC. An example of the three tumor partition methods is illustrated in Figure 2 with one high-Ki-67 case and one low-Ki-67 case. More detailed descriptions of the tumor partitioning methods are provided below.

Peak enhancement rate (PER)

The PER was evaluated for every voxel, which represents the peak relative enhancement. More specifically, the relative difference in each registered pixel value between image series was calculated using the following equation:

$$\mathrm{PER}(i) = \max\left\{\frac{I_T(i) - I_0(i)}{I_0(i)}\right\}, T = \{1,2,3,4,5\},$$

where $I_T(i)$ represents the value of the *i*th-matched pixel in the *T*th image scan (i.e., S_T). The PER for each voxel at all slices was categorized into three subgroups, which indicated mild (0 - 100%), moderate (100 - 150%) and high (>150%) enhancement, respectively, using the subtraction of precontrast and postcontrast T1-weighted fat-suppressed images, similar to the method performed in a previous study for the evaluation of background parenchymal signal enhancement ratios (33). Therefore, we divided breast tumors into three subregions with different levels of PER (Figure 2).

Time to peak (TTP)

The TTP for every voxel at all slices within the tumor represented the time at which peak enhancement was achieved, which was defined with the following equation:

$$\mathrm{TTP}(i) = \underset{T}{\operatorname{argmax}} \left\{ \frac{I_T(i) - I_0(i)}{I_0(i)} \right\}, T = \{1,2,3,4,5\},$$

where the definition of $I_T(i)$ is the same as shown above. We then partitioned the voxels within the tumor based on their TTP values. More specifically, pixel sets at the third, fourth and fifth series of postcontrast MR images to achieve peak enhancement values were defined as early, moderate and late TTP, respectively, which was similar to the method described in a previous study (27). The tumor was therefore partitioned into three regions representing various extensions of TTP value.

Kinetic pattern clustering (PKC)

To fully take advantage of features from kinetic curves, we categorized each pixel at all slices in the breast tumor by KPC according to the signal of all the postcontrast series. In particular, unsupervised K-means clustering was used to partition the whole tumor into several spatially distinct subregions, where tumor voxels with similar enhancement patterns were grouped together (29). For each pixel, the enhancement rates in all five postcontrast series were calculated, as shown in the following equation:

$$R_T(i) = \left\{ \frac{I_T(i) - I_0(i)}{I_0(i)} \right\}, T = \{1,2,3,4,5\},$$

where the definition of $I_T(i)$ is the same as shown above. The similarity between each pair of pixels was measured by the Euclidean distance between the vectors, which were obtained from the five enhancement rates (i.e., $R_T(i)$). The tumor was therefore segmented using FCM on tumor voxels of DCE-MRI. To determine the number of

subregions (i.e., the cluster number K), we set K from 2 to 5 and selected the one with the optimal Calinski-Harabasz value which measures ratio of between-cluster variance and within-cluster variance (34). Each breast tumor was therefore divided into regions composed of the highest, moderate and lowest levels of overall enhancement rate.

Feature extraction

Texture features were assessed using the gray level co-occurrence matrix (GLCM) based on 3D analysis to reflect the shape and/or spatial complexity of the tumor or tumor subregions (35). Due to the exploratory nature of this study, a wide array of volumetric texture features were computed based on the GLCM (Table 1). From the registered MRI sequential scans, 18 texture features were extracted on the precontrast, the mediate (i.e., the third) and the last (the fifth) postcontrast sagittal fat-suppressed T1-weighted MR image sequences, which were termed S-0, S-1 and S-2, respectively. We included precontrast image sequence for analysis because it was previously found for association with molecular subtypes of breast cancer (16, 17).

Statistical analysis

Differences in categorical variables (menopausal status, family history and tumor type) between molecular subtype characteristics were assessed using the χ^2 test or Fisher's exact test if the expected frequency in any cell of the table was less than five. Statistical differences in tumor volume between the histopathology groups were evaluated by the student's t-test.

Both univariate and multivariate logistic regression analyses were performed to assess the predictive power of individual predictors and their complementary value for

classifying tumors into high- or low-Ki-67 expression groups. To reduce the impact of data co-linearity on the statistical model for classification, features with similarity (i.e., correlation coefficient) greater than 0.8 to other features were removed. For each of the two features with higher similarity, the average correlations with the other features were calculated, and the feature with the higher correlation was eliminated.

To avoid overfitting of classifiers, a leave-one out cross-validation (LOOCV) method was used for the statistical model. In each loop of the LOOCV, one sample was retained as the test case, and the other samples were used as the training set. At each LOOCV loop, the feature selection procedure of the stepwise logistic regression model was applied on the training set to produce the optimal subset of features for prediction. The procedure was repeated for all the LOOCV folds, and a classification score was generated for each test case. The importance of image features was evaluated by counting the number of times they were selected over all of the LOOCV loops.

The predictive capability of the proposed imaging predictors was assessed using a receiver operating characteristics (ROC) curve analysis and the area under the curve (AUC). To control the false discovery rate (FDR), the Benjamini-Hochberg method was used to adjust for multiple statistical testing (36). AUCs were compared using a bootstrap test implemented in the pROC package of R program (37). $P < 0.05$ was considered statistically significant. All statistical analyses were performed in Matlab version R2015a (The MathWorks Inc., Natick, US) and R version 3.3.2 (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Subjects

Patients with Ki-67 expression levels more than 14% represented 51 (66.2%) of all samples. Statistical tests showed no significant associations between the molecular subtypes and the pathologic conditions (Table 1). There were no significant differences in tumor volume (p=0.779) between the high- and low-Ki-67 proliferation status.

Univariate analysis of image features in tumor subregions

The performance (in terms of AUC) of each individual feature in the entire tumor was evaluated using univariate regression classifiers (Table 3). However, these results indicated relatively lower discriminative power compared to analysis of subregions.

Table 3 also shows the three best features in various tumor subregions according to the tumor segmentation methods based on TTP, PER and KPC. Among all regions, the areas with high PER and early or moderate TTP showed the highest performance, in which the best three individual features had AUC values ranging from 0.700 to 0.750. The best single feature was given by the texture statistic of sum variance in the S-1 image sequence on the tumor subregion related to the early TTP value, which yielded an AUC of 0.748 and 95% confidence intervals (CI) from 0.639 to 0.857 (Figure 4 and Table 3). Figures 2 and 3 shows the examples of the best performance feature, i.e., sum variance and cluster prominence which obtained from tumor subregion related with early TTP and high KPC, respectively. We observed a relatively large difference of this feature between the low- and high-Ki-67 tumors, while some less successful cases were also observed (Figures 2 and 3). The performance (in terms of AUC) of each tumor subregion was compared to that of the entire tumor values (Table 3). The results showed

that individual features extracted from early and moderate TTP areas had significantly better performance (corrected p-value less than 0.05) than those from the whole tumor.

Multivariate analysis of intratumoral image features to predict Ki-67 status

From each partitioned tumor subregion, AUC under ROCs for a multivariate logistic regression classifier with a LOOCV test along with a comparison against the entire tumor were shown in Table 5. The ROC plots based on classifiers with features from various tumor subregions are shown in Figure 5. In tumor areas related to early TTP, a multivariate logistic classifier achieved an AUC of 0.807±0.050, which was the best performance classifier among the three regions, and was significantly higher ($p = 0.0002$) than that based on the entire tumor (Table 4). In addition, classifiers in tumor subregions related to a high PER level showed AUCs of 0.722 and 0.721 on MR S-1 and S-2 image series, respectively, which are higher than that of the other regions (Table 4). Finally, the tumor region based on KPC associated with the highest overall voxel enhancement on MR series S-0 yielded an AUC of 0.731, which was the highest among that of the three regions and was significantly higher than that based on the entire tumor ($p = 0.0084$).

For each tumor partitioning method, the selection frequencies of each image feature in multivariate classifiers with the best performance among the three subregions are shown in Table 5. In the predictive model with early TTP tumor area, most (5 out of 6) selected features (i.e., cluster prominence, cluster shade, correlation, maximum probability and difference entropy) were chosen with a high frequency of more than 96%, while the other feature, namely, inverse difference, was selected only once over

the 77 loops. Tumor partitioning based on TTP values indicated higher performance and a more stable classifier compared to the other tumor partition methods.

DISCUSSION

In this work, a comprehensive analysis of tumor subregion partitioning methods was conducted to identify intratumoral regions, in which imaging GLCM texture features reflecting the spatial heterogeneity of the DCE-MRI signal were utilized for the prediction of Ki-67 status in ER-positive breast cancer. We found significant correlations between texture features in tumor subregions and Ki-67 proliferation status, in which the tumor region associated with early TTP showed the highest performance for prediction. Our findings suggested that image features extracted in areas that are related to the most aggressive phenotype of the tumor, rather than the whole tumor, play a more important role in discriminating tumor characteristics (27).

Previous studies have reported that contrast enhancement patterns in intratumoral regions correlated with clinical and histologic features (27-29). A related study by Chaudhury et al (26) showed that intratumoral regions with rapid gadolinium washout were associated with the ER status and nodal metastasis. A recent study identified intratumoral heterogeneity features for discriminating ER status, HER2 status, and triple-negative molecular subtypes (30). In our study, a comprehensive analysis of tumor partitioning methods was conducted in which the performance was compared to identify the effectiveness of these methods. The high temporal resolution may facilitate a finer partitioning of tumor subregions for the tumor partitioning methods. Moreover, high spatial resolution can help to more accurate texture analysis. It is interesting to

note that the classifiers with relatively higher performance were observed in tumor subregions related to high PER, early TTP, and high overall enhancement rate. A possible explanation is that these tumor subregions potentially reflect angiogenesis, which could be more indicative of aggressive tumor-related Ki-67 proliferation status (19, 38).

We included precontrast MR series for tumor characterization. It is interesting to note that, for tumor subregions based on PER or TTP, the best classifier performance was provided on the postcontrast MR series, whereas for tumor subregions based on KPC, the best performance classifier was obtained at the precontrast MR series (Tables 1 and 3). Related studies (16, 29) examined the utility of GLCM based texture features for predicting breast tumor molecular subtypes or pathological response of breast cancer to Neoadjuvant Chemotherapy. In addition to the texture features utilized in those studies, we explored more features such as cluster prominence and cluster shade, which are among the best performance for associating with Ki-67 status.

In this study, the GLCM texture statistics of sum variance and cluster prominence from the early TTP area were among the best-performing individual features, in which a higher level of these features was observed in lower-Ki-67 patients than in high-Ki-67-expressing patients. The individual texture feature of cluster prominence is a measure of asymmetry; if the cluster prominence value is high, the image is less symmetric (39). Additionally, this feature was among the best in the tumor subregions related to the highest overall enhancement rate based on the KPC method. For both the tumor partitioning methods of TTP and KPC, this feature showed a selection frequency

of 100% during the LOOCV loops, which was significantly better in terms of the AUC value than that obtained for the entire tumor. Moreover, the difference entropy measures image heterogeneity, reflecting randomness of the difference of neighboring voxels' gray-levels. This feature have relatively high performance for the tumor partition methods of PER, and was selected with high frequency for partitioning methods of TTP, and PER, respectively. These texture features, which cannot be accurately and reliably evaluated using a visual or subjective evaluation method, could be used as candidate biomarkers for associations with biologic characteristics of the tumor.

Our study has several limitations. This study was retrospective in nature, and the statistical power was limited by the relatively small number of samples. A texture analysis of DCE-MRI data was performed, while first-order statistical features such as skewness and kurtosis and other second-order texture statistics based on gray-level run-length matrix (GLRL) (26) were not included in this study, which might provide characteristics of the tumor. Further prospective investigations are needed to validate the clinical utility of our work.

In conclusion, intratumoral partitioning identified breast tumor subregions related with early TTP in which DCE-MRI parameters were used as predictors for discriminating Ki-67 proliferation status in breast cancer. Further work is needed before these quantitative MRI parameters could be used to facilitate the noninvasive assessment of characteristics of breast cancer in clinical practice.

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Tables

Table 1. Tumor characteristics

Characteristic	All 77	Low <14% (n=26) (%)	High ≥14% (n=51) (%)	P-value[a]
PR[b]				0.766
Positive	65	21(27.3)	44(57.1)	
Negative	12	5(6.5)	7(9.1)	
Histopathology[c]				0.507
Invasive ductal	63	21(27.3)	42(54.5)	
Invasive lobular carcinoma	2	0(0)	2(2.6)	
Mixed	12	5(6.5)	7(9.1)	
Menopausal status[c]				0.410
Premenopausal	8	4(5.2)	4(5.2)	
Postmenopausal	60	18(23.4)	42(54.5)	
N/A	9	4 (5.2)	5 (6.5)	
Age (y)	53.3(27–71)	54.9(38–71)	52.44(27–67)	0.260
Maximum tumour diameter (mm)	25.8 (10–70)	27.5 (10–70)	24.2 (10–50)	0.336

[a]P-value for low-Ki-67 versus high-Ki-67 comparison.

[b]Data were tested using the Chi-square test.

[c]Data were tested using Fisher's exact test.

PR, progesterone receptor.

N/A, not avaiable

Numbers in parentheses are percentages.

Table 2. Texture feature description

Feature	Description
Autocorrelation	Detect repetitive patterns of texture elements
Cluster Prominence	Asymmetry
Cluster Shade	Asymmetry or uniformity
Correlation	Image complexity
Contrast	Local variations presented in an image
Difference Entropy	Randomness of the difference of neighboring voxels' gray-levels
Dissimilarity	Local contrasts
Difference variance	Variations of difference of gray-level pairs
Entropy	Randomness of the image texture (intensity distribution)
Energy	Homogeneity of an image
Homogeneity	Closeness of the distribution of elements in the GLCM to the GLCM diagonal
Inverse Difference (moment)	Local homogeneity
Information correlation 1	Nonlinear gray-level dependence
Information correlation 2	Nonlinear gray-level dependence
Maximum Probability	Highest frequency of pixel pair
Sum average	Overall brightness
Sum variance	Spread in the sum of the gray-levels of voxel-pairs distribution
Sum entropy	Randomness of the sum of gray-levels of neighboring voxels

Table 3. Univariate analysis of texture-related imaging features for predicting Ki-67 status

Method	Subregion	Feature	AUC	Interval	P-value*
PER	High	Difference Variance (S-2)	0.714	0.598-0.831	0.096
		Difference Entropy (S-2)	0.711	0.591-0.832	0.577
		Difference Variance (S-1)	0.706	0.586-0.825	0.213
	Moderate	Homogeneity (S-2)	0.649	0.511-0.786	0.744
		Difference Variance (S-1)	0.638	0.503-0.773	0.714
		Inverse Difference (S-2)	0.638	0.501-0.775	**<0.0001**
	Low	Difference Variance (S-0)	0.669	0.515-0.796	0.619
		Difference Variance (S-1)	0.620	0.485-0.755	0.932
		Difference Variance (S-2)	0.598	0.463-0.733	0.685
TTP	Early	Sum variance (S-1)	0.748	0.639-0.857	**0.047**
		Cluster Prominence (S-1)	0.733	0.614-0.852	**0.006**
		Sum variance (S-2)	0.715	0.597-0.833	0.514
	Moderate	Information Correlation 2 (S-1)	0.720	0.594-0.847	**0.004**
		Information Correlation 2 (S-2)	0.713	0.586-0.841	**0.027**
		Information Correlation 1 (S-1)	0.701	0.562-0.841	**<0.0001**
	Late	Difference Entropy (S-2)	0.647	0.514-0.780	**<0.0001**
		Difference Entropy (S-1)	0.627	0.491-0.762	0.289
		Sum variance (S-1)	0.623	0.485-0.761	0.349
KPC	High	Cluster Prominence (S-0)	0.731	0.624-0.853	**<0.0001**
		Sum variance (S-0)	0.724	0.611-0.838	**0.001**
		Difference Variance (S-0)	0.667	0.542-0.793	0.707
	Moderate	Difference Variance (S-0)	0.710	0.590-0.830	0.073
		Dissimilarity (S-0)	0.689	0.564-0.813	0.143
		Contrast (S-0)	0.683	0.557-0.808	0.123
	Low	Difference Entropy (S-2)	0.652	0.525-0.780	0.684
		Difference Variance (S-2)	0.646	0.518-0.773	0.695
		Correlation (S-2)	0.620	0.443-0.719	0.695
Entire tumor		Inverse Difference (S-2)	0.665	0.513-0.793	/
		Inverse Difference (S-1)	0.664	0.513-0.792	/
		Homogeneity (S-1)	0.650	0.511-0.789	/

*FDR-corrected p-values for comparisons of individual classifier performances between features from tumor subregions and the corresponding features from the entire tumor.

S-0, S-1 and S-2 represent precontrast, the third- and the fifth- postcontrast MR series, respectively.

PER, Peak enhancement rate.

TTP, Time to peak.

KPC, Kinetic pattern clustering.

Table 4 Multivariate analysis of texture-related imaging features for predicting Ki-67 status

Partition method	Subregion	S-0		S-1		S-2	
PER		AUC	p-value	AUC	p-value	AUC	p-value
	High	0.636±0.067	0.012	**0.722±0.065**	**0.005**	0.721±0.063	0.008
	Moderate	0.518±0.070	0.831	0.605±0.072	0.332	0.502±0.072	0.468
	Low	0.658±0.066	0.013	0.444±0.070	0.876	0.411±0.072	0.918
TTP							
	Early	0.635±0.074	0.153	**0.807±0.050**	**0.0002**	0.732±0.061	0.041
	Moderate	0.672±0.072	0.022	0.718±0.066	0.013	0.701±0.066	0.049
	Late	0.496±0.071	0.931	0.535±0.072	0.965	0.587±0.071	0.939
KPC							
	High	**0.731±0.060**	**0.0008**	0.617±0.065	0.174	0.615±0.070	0.541
	Moderate	0.650±0.069	0.032	0.623±0.065	0.115	0.629±0.067	0.455
	Low	0.300±0.077	0.049	0.606±0.066	0.189	0.586±0.068	0.929
Whole tumor							
		0.502±0.068		0.537±0.068		**0.590±0.068**	

S-0, S-1 and S-2 represent precontrast and the third and fifth postcontrast series, respectively.

PER, Peak enhancement rate.

TTP, Time to peak.

KPC, Kinetic pattern clustering.

Table 5. List of texture features that were selected in LOOCV loops for the best performance classifier in each tumor partition method

Partition method	Feature	Selection Frequency
PER	Information Correlation	76 (98.7%)
	Difference Entropy	59 (76.6%)
	Entropy	15 (19.5%)
	Information Correlation	2 (2.6%)
	Inverse Difference	2 (2.6%)
	Dissimilarity	1 (1.3%)
TTP	Cluster Prominence	77 (100%)
	Cluster Shade	77 (100%)
	Correlation	75 (97.4%)
	Maximum Probability	75 (97.4%)
	Difference Entropy	74 (96.1%)
	Inverse Difference	1 (1.3%)
KPC	Cluster Prominence	77 (100%)
Whole Tumor	Inverse Difference	62 (80.5%)
	Entropy	6 (7.8%)
	Dissimilarity	5 (6.5%)
	Cluster Shade	4 (5.2%)
	Difference Entropy	4 (5.2%)

PER, Peak enhancement rate.

TTP, Time to peak.

KPC, Kinetic pattern clustering.

Figure legends

Figure 1. Illustration of intratumor partitioning results of one high-Ki-67 and one low-Ki-67 case. The first row shows examples of one high-Ki-67 case and the corresponding kinetic curves (from a to d), while the second row shows one low-Ki-67 tumor with the corresponding kinetic curves (from e to h). The first column shows segmented tumors and their kinetic curves (a, e). The second column shows tumor subregions partitioned by PER with the red, green and blue colors representing high, moderate and low levels of PER, respectively (b, f). The third column shows tumors partitioned by TTP with the red, green and blue colors representing early, moderate and late TTP, respectively (c, g). The fourth column shows tumor partitioning by KPC with the red, green and blue colors representing high, moderate and low levels of overall enhancement rates, respectively (d, h).

Figure 2. Representative texture feature of sum variance obtained from three tumor subregions. Tumors are partitioned by TTP with the red, green and blue colors representing early, moderate and late TTP, respectively. The sum variance obtained in subregion related with early TTP showed a much higher value in Low-Ki-67 tumor (a) compared to that in High-Ki-67 tumor (b), while a similar value of this feature was also observed in a low (c) and high-Ki-67 tumors.

Figure 3. Representative texture feature of cluster prominence obtained from three tumor subregions. Tumors are partitioned by KPC with the red, green and blue colors

representing high, moderate and low KPC, respectively. This feature obtained in tumor subregion related with high KPC showed a much higher value in Low-Ki-67 tumor (a) compared to that in High-Ki-67 tumor (b), while a similar value of this feature were also observed in a low (c) and high-Ki-67 tumors.

Figure 4. Boxplot graphs reveal statistically significant differences between the values of the three best features. (a) Sum variance in the S-1 series for the TTP map. (b) Difference variance in the S-1 series for the PER map (c). Difference Variance in the S-2 series for the KPC map.

Figure 5. Comparison of ROC plot based on tumor subregions and the entire tumor.