Local fuzzy c-means clustering for medical spectroscopy images.

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Abstract

In this paper we use a local fuzzy c-means clustering for analysis of data from spectroscopy which allows validate the hypothesis of the action clioquinol (CQ), a new drug for prostate tumors. But even through the algorithms proposed, it is possible to see that the action of the drug depends on the concentration of copper in tissue, which is known by previous studies to be higher in tumor tissue than in healthy subjects.

Key words: Prostate cancer, Fuzzy c-means clustering (FCM), Image segmentation

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1. Introduction

Recently, organic copper complexes has been report as proteasome inhibitors and apoptosis inducers [2].

Apoptosis is a highly conserved cellular suicide program in multicellular organism from worms to human. This cellular death program serves as a means to maintain multicellular organisms by discarding and damaged and undesirable cells [5].

It has been suggested that cancer cells are more sensitive to several apoptosis-inducing stimuli than normal cells, including proteasome inhibitors [4].

The proteasome is a intracellular protease, and its functions in cells are a variety of important intracellular events, including cell cycle progression, antigen-presenting pathway and apoptosis [1].

The antitumor activity of proteasome has been confirmed by the results of Phase I and II trials using PS-341, however some side effects were observed suggesting that there is a need to discover a novel protesome inhibitors with no, or much less, toxicity [7].

The ubiquitin-proteasome pathway is the principle pathway for intracellular protein degradation and it plays a significant role in neoplastic growth and metastasis.

Nuclear factor κB (NF-κB) is a major transcription factor that plays an essential role in several aspects of human health. The desregulation of NF-κB is associated with cancer for the ability of NF-κB to suppress apoptosis [8].

The ubiquitin-proteasome pathway is requered for activation of NF-κB by degradation of its inhibitory protein, IκB.

Inhibition of proteasome mediated IκB degradation may limit tumor growth and metastasis make cancer cells more sensitive to apoptosis [9].

The growth of new blood cells is a process called angiogenesis. Antiangiogenesis involves the hypothesis that cancer may be stopped by depriving tumors of the blood supply that nourishes them [11].

Angiogenesis, the neovascularization process, involves a sequence of multiple events of vascular endothelials cells, including differentiation, acquisition of migrative and proliferative abilities with high matrix-degrading activity, and tube formation of cells.

Tumor growth and metastasis depend upon angiogenesis, that requires growth factors, proteases, and trace element copper. High levels of copper have been found in many types of human cancers [6].

Copper stimulates proliferation and migration of human endothelias cells and is requered for the secretion of several angiogenic factors by tumor cells ([3], [10].

In addition , there is increasing evidence suggesting a correlation between apoptosis and angiogenesis. For example, some angiogenesis inhibitors, including angiostatin and endostatin, induce dormancy of primary tumors and metastases by indirectly increasing apoptosis in tumor cells.

There is evidence that copper chelation acts through inhibition of NF-κB activity in cancer cell lines although the molecular mechanism has not been show [12].

Clioquinol (CQ) is a lipophilic compound of quinoline class that is capable of forming stable complexes with copper(II) ions [14]. CQ is a well tolerated copper binding compound and CQ-copper complexes is a potent proteasome inhibitor and apoptosis inducer in tested prostate and breast cancer.

In this work we proposed an algorithm for analysis of data from spectroscopy which allows validate the hypothesis of the action Cu and CQ in prostate tumors.

But even through the algorithms proposed, it is possible to see that the action of the drug depends on the concentration of copper in tissue, which is known by previous studies to be higher in tumor tissue than in healthy subjects.

2. Materials and Methods.

2.1. Data Description

Four samples were measured under the following conditions. X-ray focused Beam (5 mx5 m beam size using a KB mirrors system , 10.0 keV) was used for fluorescence excitation of the samples. The geometrical arrangement was the standard, i.e., the sample is mounted at 45 degree angle relative to the beam path and the detection system is at 90 degrees. A Ketek silicon drift detector 100 mm^2 active area was used for fluorescence detection. The intensity of the micro x-ray beam was monitored using a small ion chamber mounted downstream the KB mirrors in front of

the sample. The samples were mounted in an XYZ positioner, which allows moving the sample with 0.1 m accuracy in any direction. The scans were performed in both vertical and horizontal directions to cover the entire size of the sample by the excitation beam, using a step size of 5 microns in both directions. The acquisition time was 1 seconds per point. The data was analyzed using a Matlab code written for this purpose. The fluorescence counts were normalized by the intensity of the incoming beam.

Figure (1) represents the elemental distribution of Ca, Fe, Cu and Zn from sample C4-Normal. The numbers on each axis represent the actual point position in millimeters. The fluorescence intensities are plotted using a color code that is shown on the left of each picture. The minimum and maximum values were selected to enhance the contrast between background signal and fluorescence signal. The spots having the maximum value or higher are shown in red. The color code range for the elements was the same in all pictures for comparison purposes.

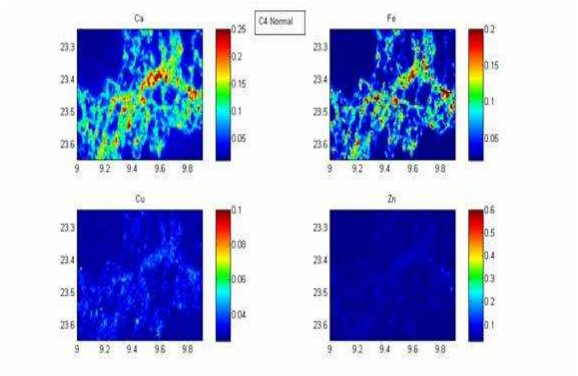

Figure 1: Distribution Normal Tissue

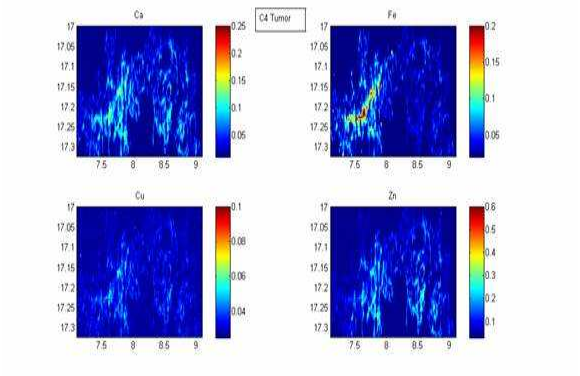

Figure 2: Distribution Untreated Tumor

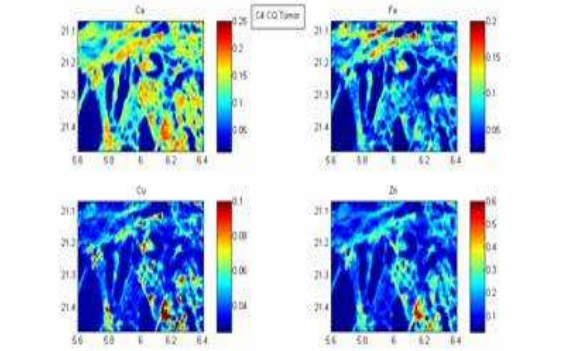

Figure 3: Distribution Treated Tumor

2.2. Local Fuzzy C-means algorithms

Fuzzy C-means (FCM) is one of the most used methods for image segmentation and its success chiefly attributes to the introduction of fuzziness for the belongingness of each image pixels.However, one disadvantage of standard FCM is not to consider any spatial information in image context.

Recently, many research have incorporated local spatial information into the original FCM algorithm to improve the performance image segmentation [16],[17],[18].

Given a set of data $\{x_1, ..x_N\}$, one can raise the problem of separating them into K sets, so that each group representing a particular condition among the data that they belong to, this problem is called clustering. There are many algorithms to do this, in this case we consider a generalization of the well-known C-means algorithm.

In the algorithm C-means we consider prototypes vectors (centers) $C_1, ..C_K$ which are the representatives of the K groups.

The components of these vectors are variables and they minimized the following functional:

$$J(C) = \sum_{i=1}^{K}\sum_{j=1}^{N} d^2(x_j, C_i).$$

Now, we consider the following generalized functional:

$$J_m(C) = \sum_{i=1}^{K}\sum_{j=1}^{N} (u_{ji})^m d^2(x_j, C_i),$$

where the element u_{ji} represents the probability of data x_j to belong to the group i. Of course,we need the following condition:

$$\sum_{i=1}^{K} u_{ji} = 1,$$

and the parameter m is called the parameter of fuzziness, chosen equal to two in this case.

The algorithm earlier is not designed initially for images, as it does not consider the local distribution (spatial) of data, two vectors equal will be equal regardless of their location space for resolve this the function is penalized with a term that takes into account this distribution as follows:

$$\begin{aligned} J_m(C) &= \sum_{i=1}^{K}\sum_{j=1}^{N} (u_{ji})^m d^2(x_j, C_i) \\ &+ \alpha \sum_{i=1}^{K}\sum_{j=1}^{N} (u_{ji})^m d^2(\overline{x_j}, C_i). \end{aligned}$$

where $\overline{x_j}$ is the average in a window determined by the user.

3. Results and Discussion

The idea is to implement this algorithm to the maps of distribution of Ca, Cu, Fe and Zn obtained by spectroscopy of normal tissue, untreated tumor tissue and tumor tissue treated with the drug. This will form the vectors

$$x_k = (Ca, Cu, Fe, Zn),$$

where the image is read from top to bottom and from left to right for the position k and placed in the vector concentrations given by the image at that point.

First, we project the data on the coordinates Cu, Fe and Zn and to observe a possible separation of these two groups; which is not observable. By applying the algorithm to data to separate them into two groups and taking for each concentration of the highest probability of copper being in a group (matrix U) there is a separation almost disjunta value in 0.022 for the treated tissue. In the region intersection probability of being in a group or the other this at $[0.49, 0.51]$. For the untreated tissue the separation is almost the same but the threshold is less than 0,022 For the normal tissue there is no evidence of the separation in two groups have a bearing on the amount of copper, i.e. the intesection is great, moreover the group 2 included in almost group 1. Put another way data with the same amount of copper may belong to any group with high probability.

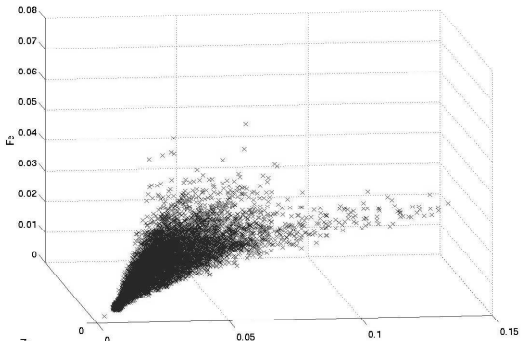

Figure 4: Projection of data

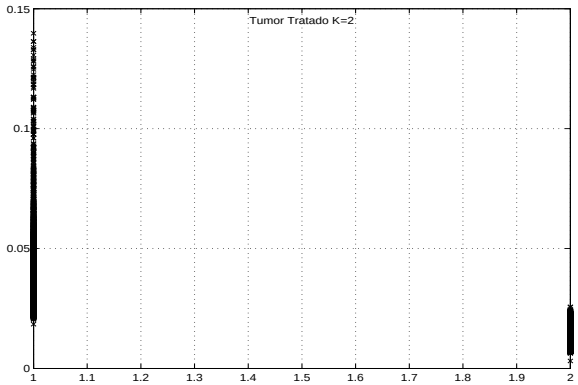

Figure 5: Separation of Cu into 2 groups - Treated Tissue

In the following three graphics viewing the separation of other metals

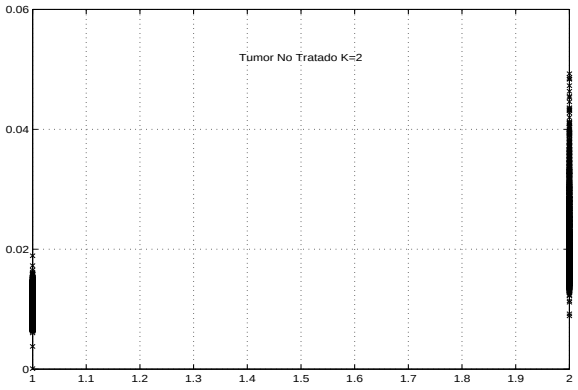

Figure 6: Separation of Cu into 2 groups - Untreated Tissue

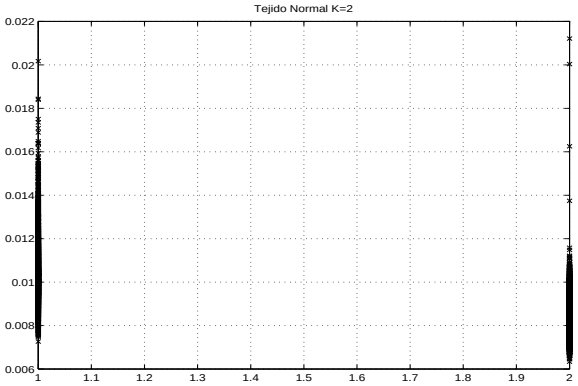

Figure 7: Separation of Cu into 2 groups - Normal Tissue

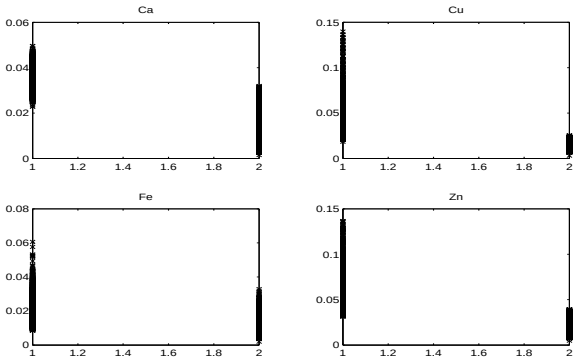

Figure 8: Separation of the four metals into 2 groups- Treated Tissue

In the case of the treated tissue and untreated tissue the separation into two groups is directly related to the concentration of copper, which in principle was not obvious, and gives indications of existence of a concentration threshold that separates two types of processes and two kinds of cells (healthy and diseased?)

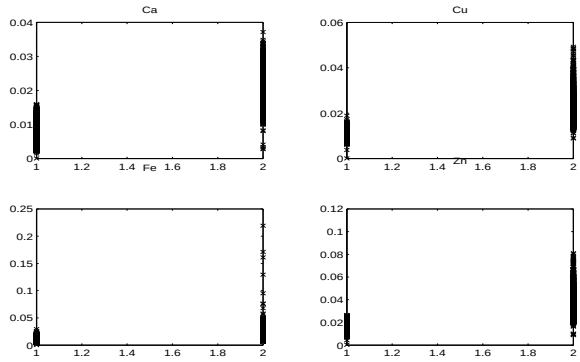

Figure 9: Separation of the four metals into 2 groups- Untreated Tissue

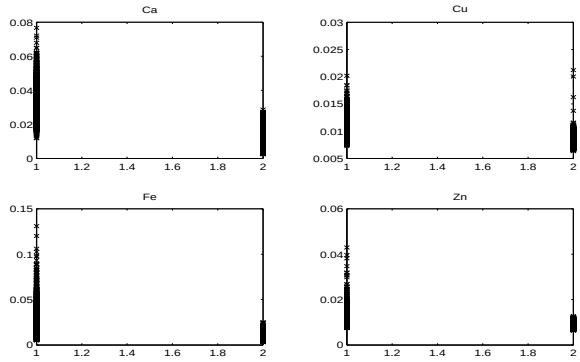

Figure 10: Separation of the four metals into 2 groups - Normal Tissue

4. Conclusions

In this paper a local fuzzy clustering method is proposed for obtain a characterization of the action of a new drug in prostate cancer. The method allows to observe the action of the drug depends on the concentration of copper in tissues, a hypothesis that emerged from previous clinical trials. This algorithm is simple and allows to analyze images obtained by spectroscopy of tissues treated with the drug, the results are easy to interpret and allow them to support the clinical hypothesis

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