Probabilistic Grading of Intracranial Gliomas in Digital Microscope Images Based on EGFR Quantity*

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ABSTRACT

A glioma is a type of cancer occurring, in the majority of cases, in the brain. The World Health Organization (WHO) assigns a grade from I to IV to this tumor, with I being the least aggressive and IV being the most aggressive. In glioma cells of grade IV the Epidermal Growth Factor Receptors (EGFRs) are over expressed. In this paper we hypothesize that this overexpression occurs also for gliomas of grades I to III. Moreover, we present a medical study aiming to determine the correlation between the WHO classification and the EGFR quantity in glioma tissue. We define four quantity classes for EGFR. First, results of immunohistochemical staining on brain glioma slices, which visualize the EGFR quantity, are examined under an optical microscope and manually classified into these four classes by a medical expert. In this paper we propose to perform this classification automatically using statistical pattern recognition technique for digital images. For this, digital microscope images of glioma are acquired and their histograms computed. Afterwards, all four EGFR quantity grades (image classes) are statistically modeled using training samples. This allows a fully automatic classification of unknown images into one of the four classes using the Maximum Likelihood (ML) estimation. Experimental results show that, on the one hand, the automatic EGFR quantity grading performs nearly with the same accuracy as the manual analysis by a medical expert, on the other hand, it is done much faster.

Keywords: Intracranial Cancer, Glioma, Immunohistochemistry, EGFR, Image Histogram, Statistical Image Classification

1. INTRODUCTION

Incidence of intracranial cancers varies around 7 to 10 per 100 thousand people in a year, with glioma being the most frequent of them. The World Health Organization (WHO) assigns a grade from I to IV to this malignant cancer, with I being the least aggressive and IV being the most aggressive. 15% to 20% of intracranial cancers are glioblastomas (glioma grade IV) with a five-year survival rate of only 3%.[1] For this reason, a high quality research leading, as soon as possible, to better understanding of the genesis and progression of glioma is highly desirable. In this paper we present a medical study (Section 2) aiming to determine the correlation between the WHO classification and the quantity of Epidermal Growth Factor Receptors (EGFRs) in glioma tissue. Commonly, the tissue slices are examined under an optical microscope. However, their digital images can also be acquired and analyzed with computer-based image classification techniques.

In Section 3, an image classification algorithm is described which allows to perform the grading of the digital microscope images in a fully automatic and very robust way. Since probabilistic approaches turned out to be appropriate to this kind of scientific problems,[2] we use the so called Maximum Likelihood (ML)[3] estimation for

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image classification. In our medical study, the number of training images for all classes (EGFR quantity grades) is quite high. Furthermore, feature values computed from the images behave very regularly and are be modeled by normal density functions.

In order to evaluate the automatic classification technique, an annotated image database (ground truth) has been created by a medical expert. Using this ground truth the classification accuracy of our automatic algorithm has been determined and is presented in Section 4.

Section 5 closes the paper with some final conclusions and a brief outline for the future work.

2. EGFR DETECTION IN GLIOMA BY IMMUNOHISTOCHEMISTRY

Glial cells, commonly called neuroglia or simply glia (Greek for "glue"), are non-neuronal cells that provide support and nutrition, maintain homeostasis, form myelin, and participate in signal transmission in the nervous system. In the human brain, glia are estimated to outnumber neurons by about 10 to 1. The four main functions of glial cells are to surround neurons and hold them in place, to supply nutrients and oxygen to neurons, to insulate one neuron from another, and to destroy pathogens and remove dead neurons. Glioma is a type of cancer that starts in the brain or spine. It is called a glioma because it arises from glial cells. The most common site of gliomas is the brain. To name the biological valency the World Health Organization (WHO) divides the intracranial cancers into grades from I to IV.[1] The glioma subcategories can be assigned into these four grades as follows: I - pilocytic astrocytoma, II - diffuse astrocytoma, III - anaplastic astrocytoma, IV - glioblastoma multiforme.

Cancer growth is supported by two main systems, namely oncogenes and suppressor genes.[4] In this paper we concentrate on the first ones. Oncogenes are physiological cellular genes. Their expression products regulate proliferation, mobility, and differentiation of cells. Furthermore, the oncogenes are responsible for the production of oncoproteins which are components of a complex network of intracellular signal transition. In result, mitotic cell division takes place. In physiological cells the growth stimulus is activated exclusively by a receptor ligand binding. For genesis and progression of cancer, oncogenes play a central role. On the one hand, mutation of oncogenes leads to proteins with disregulation and constitutive activation, i. e, no ligand is required for this activation. On the other hand, activated oncogenes lead to overexpression of physiological oncoproteins which ends up with the hyperfunction of the signal transition. In both cases we speak about the so called "gain of function".[5] From a high diversity of cellular oncogenes we pay our attention to the growth factor receptors, more specifically to the Epidermal Growth Factor Receptor (EGFR).

In our medical study we aim to determine the correlation between the quantity of EGFRs and the WHO classification of glioma. In order to do so, the Immunohistochemistry (IHC) is applied. The IHC uses the specificity and affinity of antibodies to their antigenes[†], in our case EGFR. The IHC method consists of two steps. In the first step, a primary antibody detects specific epitope of the searched antigene in the cell. In the second step, secondary antibodies are used. They become visible by an enzyme reaction and obtain the green color. Subsequently, we examine the tissue slices under an optical microscope and classify them into four categories (EGFR quantity grades) based on the intensity of the green color. This classification process is done for hundreds of microscope slides which is very time consuming.

3. PROBABILISTIC INTERPRETATION OF MICROSCOPE IMAGES

Digital microscope images representing the results of immunohistochemical staining on brain glioma slices are analyzed in this section. As mentioned in Section 2, green areas on the microscope slides indicate the occurrence and represent the quantity of the EGFRs. The intensity of this green areas is a crucial visual feature for classifying the brain glioma into one of the four EGFR quantity grades $\Omega_{\kappa=1,\ldots,4}$ (image classes) introduced in our medical study (Section 2). First, the modeling of the four image classes based on a sample training data annotated by a medical expert is performed (Section 3.1). Second, the image class (EGFR quantity grade) $\Omega_{\widehat{\kappa}}$ for a new sample image is automatically determined in the classification phase (Section 3.2). The overall schema of the whole process is depicted in Figure 1.

[†]In our experiments we use antibodies produced by the companies Dako and Acris.

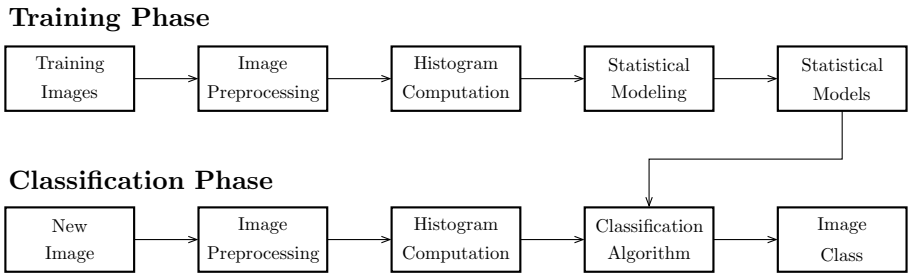

Figure 1. The processing chain presenting the training and the classification steps for EGFR quantity grading.

3.1 Training of Microscope Image Classes

First, a training set of T_κ sample images $\boldsymbol{f}_{\kappa,t=1,\ldots,T_\kappa}$ for each image class Ω_κ is acquired and annotated by a medical expert. Then, in the preprocessing step, the original RGB training images are divided into the red, the green, and the blue channels. Exclusively the green channels $\boldsymbol{g}_{\kappa,t=1,\ldots,T_\kappa}$ are taken into consideration for further modeling. Now, one dimensional histograms[6] for all images are produced, whereas the whole range of pixel values $\{0,1,\ldots,255\}$ is divided into N bins. The number of bins for all images remains the same throughout the classification task. The histograms are usually expressed as discrete functions, but we prefer to interpret them as N-dimensional global feature vectors describing the images $\boldsymbol{f}_{\kappa,t}$

$$\boldsymbol{h}_{\kappa,t} = (h_{\kappa,t,1}, h_{\kappa,t,2}, \ldots, h_{\kappa,t,N})^{\mathrm{T}} \quad . \tag{1}$$

Since the number T_κ of training images $\boldsymbol{f}_{\kappa,t}$ for each image class Ω_κ is usually quite high, statistical modeling is applied for training. It has been observed that the values of the feature vector components $h_{\kappa,t,i}$ behave regularly and can perfectly be modeled by normal density functions.[7] In order to do so, the mean values $\mu_{\kappa,i}$ and the standard deviations $\sigma_{\kappa,i}$ for all feature vector elements and all image classes are computed in accordance with the well-known Gaussian formulas

$$\mu_{\kappa,i} = \tfrac{1}{T_\kappa} \sum_{t=1}^{T_\kappa} h_{\kappa,t,i} \qquad ; \qquad \sigma^2_{\kappa,i} = \tfrac{1}{T_\kappa} \sum_{t=1}^{T_\kappa} (h_{\kappa,t,i} - \mu_{\kappa,i})^2 \quad . \tag{2}$$

Therefore, each image class Ω_κ is represented by a mean value vector and a standard deviation vector

$$\boldsymbol{\mu}_\kappa = (\mu_{\kappa,1}, \mu_{\kappa,2}, \ldots, \mu_{\kappa,i}, \ldots, \mu_{\kappa,N})^{\mathrm{T}} \quad ; \quad \boldsymbol{\sigma}_\kappa = (\sigma_{\kappa,1}, \sigma_{\kappa,2}, \ldots, \sigma_{\kappa,i}, \ldots, \sigma_{\kappa,N})^{\mathrm{T}} \tag{3}$$

after the training phase. Note that the dimension of these vectors is equal to the number N of histogram bins.

3.2 Classification of Microscope Images

In order to classify an image $\boldsymbol{f}$, a histogram with N bins based on its green channel $\boldsymbol{g}$ is computed. According to Section 3.1, this histogram is interpreted as a global feature vector

$$\boldsymbol{h} = (h_1, h_2, \ldots, h_i, \ldots, h_N)^{\mathrm{T}} \tag{4}$$

describing the image $\boldsymbol{f}$. Now, for all possible image classes $\Omega_{\kappa=1,\ldots,4}$ trained as shown in Section 3.1, the comparison with the test image is performed. For this, density values $p_{\kappa,i=1,\ldots,N}$ for all feature vector elements $h_{i=1,\ldots,N}$ are computed using the trained means and standard deviations (2) following the definition of the Gaussian density function[8]

$$p_{\kappa,i} = p(h_i|\mu_{\kappa,i}, \sigma_{\kappa,i}) = \frac{1}{\sigma_{\kappa,i}\sqrt{2\pi}} \exp\left(\frac{(h_i - \mu_{\kappa,i})^2}{-2\sigma^2_{\kappa,i}}\right) \quad . \tag{5}$$

Assuming the statistical independence between the feature vector elements, the final evaluation of the test image represented by $\boldsymbol{h}$ and a hypothesis concept Ω_κ is computed with

$$p_\kappa = p(\boldsymbol{h}|\boldsymbol{\mu}_\kappa, \boldsymbol{\sigma}_\kappa) = \prod_{i=1}^{N} p(h_i|\mu_{\kappa,i}, \sigma_{\kappa,i}) \quad . \tag{6}$$

Finally, the classification result $\Omega_{\widehat{\kappa}}$ (EGFR quantity grade) is found by maximization of the density value (6) over all possible image classes represented by their index κ

$$\widehat{\kappa} = \underset{\kappa}{\operatorname{argmax}}\, p_\kappa = \underset{\kappa}{\operatorname{argmax}}\, p(\boldsymbol{h}|\boldsymbol{\mu}_\kappa, \boldsymbol{\sigma}_\kappa) \quad . \tag{7}$$

4. EXPERIMENTS AND RESULTS

Within the medical study hundreds of microscope slides visualizing glioma tissue after immunohistochemistry were evaluated under an optical microscope. This led to a comprehensive ground truth. In order to determine the performance of the automatic classification algorithm, only a subset of this image dataset was taken into consideration. For training, 50 sample images for each image class (EGFR quantity grade) were used. In the recognition phase, 200 microscope test images were automatically classified. All images were described by histograms with $N = 25$ bins. The accuracy of the probabilistic classification algorithm described in Section 3 in comparison to the manual evaluation mentioned in Section 2 amounts to 91%. The automatic classification of 100 digital microscope images takes 9.8s on a workstation equipped with Pentium 4 (2.66 GHz, 1024 MB) and is significantly faster than the manual grading by a medical expert taking about 30s for one slide.

5. CONCLUSIONS

In this paper, an interdisciplinary research work bridging the gap between medicine and computer science has been presented. It is motivated by low survival rates of intracranial glioma patients, especially those with glioblastoma multiforme. It is highly desirable to determine the factors responsible for the genesis and progression of glioma. For this reason we perform a medical study investigating the role of the Epidermal Growth Factor Receptors (EGFRs) in this process (Section 2). One of the steps in our medical research is the immunohistochemical estimation of EGFR quantity in glioma tissues by examining microscope slides. Since these slides can be acquired as digital images, we apply an algorithm for automatic statistical classification based on the Maximum Likelihood (ML) estimation (Section 3) for this. First experimental results (Section 4) indicated, on the one hand, that the automatic EGFR quantity grading performs nearly with the same accuracy as the manual analysis by a medical expert, on the other hand, it is done much faster.

Since the area of investigations in our medical study is very wide, in the future we will also use other achievements of digital image analysis. For instance, one of the crucial features for WHO grading of gliomas is the cell shape. For this, we are going to implement an algorithm for partial shape similarity.[9]

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