Clinical Observations

PREDICTION OF THE OUTCOME OF ESOPHAGEAL EPITHELIUM DYSPLASIA BY HIGH RESOLUTION IMAGE ANALYSIS

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ABSTRACT

Objective: To predict the outcome of dysplasia of esophageal epithelium by means of high resolution image analysis (HRIA). Methods: Asymptomatic adults were examined for balloon cytology of the esophagus in 1983 from Heshun Commune of Linxian County. 93 severe dysplasias and 122 mild dysplasias of the esophagus were selected. By means of an Axiomat-microscope equipped with TV-camera, 100 normal nuclei of well-preserved cells in the intermediate layer of Pap-stained squamous epithelium were randomly examined. Results: Of the 93 cytologically diagnosed severe dysplasia cases, 24, 14 and 7 progressed to carcinoma in 3, 5 and 9 years, respectively. In the other 48 cases, dysplasia remained stable or regressed to normal. The other cases were used as the control. According to chromatin features, correct diagnosis of cases was achieved by HRIA in 75.0% (18/24), 85.7% (12/14) and 85.7% (6/7) of the cases examined, respectively ($P<0.001$). Of the 122 cytologically diagnosed mild dysplasia, 16, 13 and 12 cases progressed to carcinoma in 3, 5 and 9 years, respectively. The other 81 cases remained stable or regressed to normal. Correct diagnosis was made by HRIA in 93.8% (15/16), 76.9% (10/13) and 83.3% (10/12) of the cases examined, respectively ($P<0.001$). Conclusion: Chromatin nuclear features examined by HRIA can predict the outcome of precancerous lesions and discriminate progressor from non-progressor ones. It can be used as surrogate endpoint biomarkers for the evaluation of efficiency of chemo-prevention trial.

Key words: Esophageal neoplasms, Precancerous lesions, High resolution analysis, Esophageal dysplasia, Chromatin features, Surrogate endpoint biomarkers

Esophageal cancer is a common disease with a very poor prognosis. In 1985, esophageal cancer was estimated to be the ninth most common cancer worldwide. Nearly 50% of the world's esophageal cancer occurs in China.[1] Over the past 40 years, systematic research work has been done on precancerous lesions of the esophagus, Previous studies have reported that dysplasia of esophageal epithelium is an essential step in esophageal carcinogenesis. It is already validated as a predictor of cancer incidence.[2] The conclusions suggest that for the chemoprevention of esophageal carcinoma, it is essential to treat the precancerous lesion by blocking its progression and promoting its regression to normal.[3] At present time, cancer chemoprevention is a rapidly expanding area of oncology. But it will be emphasized that clinical trials of chemoprevention require large scale of effort (thousands of subjects), long duration (5–10

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years) and the unacceptable cost (millions of dollars). It is highly desirable that early, precancerous lesions are identified which can serve as surrogate endpoints biomarkers (SEBs) for cancer incidence in chemoprevention trials and, more importantly, as targets for chemoprevention.[4] The high resolution image analysis (HRIA) may be able to appreciate very slight but consistent differences in nuclear appearance changes that are too subtle for a cytologist to observe.

MATERIALS AND METHODS

Subjects and Specimens

Participants were recruited in the spring of 1983 from Heshun Commune, Linxian County. All the 40–65-year-olds were asked to participate the esophageal balloon cytologic examinations. 93 cases of severe dysplasia and 122 cases of mild dysplasia of the esophagus were selected for this study. During the next 3, 5 and 9 years, 24, 14 and 7 cases of severe dysplasia, respectively, progressed to esophageal carcinoma. The other 48 cases remained stable or regressed to normal and were used as the control. There were 16, 13 and 12 cases of mild dysplasia that progressed to esophageal carcinoma within the next 3, 5 and 9 years, respectively. The other 81 cases remained stable or regressed to normal and were used as the control (Table 1). All the cases were Pap-stain smears.

Data Acquistion

By means of an Axiomat microscope (Zeiss, Oberkochen, Germany) equipped with a TV camera (Bosch, T1VK9B1, Stuttgart, Germany, 512×512 pixels), 100 visually normal, well-preserved intermediated squamous cell nuclei per specimen were randomly selected and digitized. Image processing was carried out using a VAX 4000–500 processor (Digitial Equipment, Maynard, Massachusetts, USA) with software written under Interactive Data Language (RSI, Boulder, Colorado, USA). The pixel distance was 0.25 μm, and the nominal grey value resolution was covered by 256 channels. The nuclei were scanned in transmission with a 100× objective (oil immersion; numerical aperture, 1.3) using an optical narrow band filter 548 nm in wavelength. Each nucleus was automatically segmented, visually controlled and interactively improved if necessary. More than 100 quantitative features (morphologic, densitometric, textural) were extracted using the extinction or optical density image, which was derived from the transmission image. A shading correction was applied. Using linear and nonlinear filtering like Roberts' gradient, Laplace transform, flat texture image, local fractal and multifractal dimensions, topologic gradient, difference in the upper and lower skeleton, and statistical features derived from runlength and co-occurrence matrix, several chromatin distribution features were calculated from the whole nucleus as well as from dark and bright regions of the nucleus. The latter were automatically discriminated using the upper and lower skeleton, which is similar to the watershed algorithm applied to the extinction image and its inversion (upper and lower skeleton).

Statistics

All statistical evaluations were done using SAS (SAS Institute, Inc., Cary, North Carolina, USA) and BMDP (Statistical Software Inc., Los Angeles, California, USA) program packages. All cells from specimens belonging to the same clinical sample were pooled, and two-class, stepwise linear discriminant analyses on the cell level were applied. From the whole feature set only those features were offered in the classification steps that have univariate significance and are not highly dependent from staining and preparation changes. Up to 10 features were selected stepwise on the basis of F-statistics. The value for the first chosen feature is the univariate one, whereas the following F-values are multivariate, reflecting the impact of the results after using this feature together with the already selected features. For each specimen, the mean of the a posteriori probability (APOP) distribution of the corresponding cells was calculated. The APOP value and the double standard error of this mean (SEM) were used for specimen classification. A specimen was classified into that class with the highest APOP value only if the mean APOP±SEM did not cut a threshold (THR) that was set as the border between the two classes. In all cases, the threshold was defined at APOP=0.5 that is, half the distance between both group means. Cases with THR $\in$ (APOP±2SEM) were called unclear. All statistical evaluations were done at the 95% level.

RESULTS

Results of 93 esophageal severe dysplasia cases by image analysis described in Table 2. 18 of 24 specimens in the progression group and 13/19 in the nonprogression group within three years were correctly classified. The cell classification rate was 75.0% ($P<0.001$) for the progression group and 68.4% for the nonprogression group (Figure 1, Table 2). In this cell classification case, the most important features were the number of dark particles (DNO), followed by relative area of dark particles (DAA) and skewness of upper skeleton (HUM3). Twelve of 14 cases in the progression group and 8/10 in the nonprogression group within five years were correctly classified. The cell classification rate was 85.7% ($P<$ 0.0001) for the progression group and 80.0% for the

nonprogression group (Figure 2, Table 2). The best feature was sum entropy of flat texture co-occurrence matrix (NC9), followed by heterogencity of chromatin (HETERO) and range of local multifractal dimension (MFRANG). The subsequent specimen classification resulted in 6/7 correct decisions and the cell classification rate was 85.7% ($P<0.001$) in the progression group within nine years. 15 of 19 specimens in the non-progression group within nine years was correctly classified and the cell classification rate was 78.9% (Figure 3, Table 2). The best feature was NC13, which is the measure of correlation of co-occurrence of flat texture, followed by long runs emphasis of runlength distribution of flat texture (RL12) and measure of correlation of co-occurrence of extinction (C012). By means of the subset of chromatin features, the totally correct specimen classification rate of 80.0% for severe dysplasia progression group (Table 2) was achieved.

Table 1. Cytology results from the esophageal balloon cytology survey, including follow-up results and number of specimens and measured cell nuclei

Diagnosis and No. of cases (cells)	Severe dysplasia	Mild dysplasia
Progression to cancer within 3 y	24 (2,722)	16 (1,796)
Progression to cancer within 5 y	14 (1,348)	13 (1,449)
Progression to cancer within 9 y	7 (755)	12 (1,337)
Nonprogression to cancer within 9 y	48 (4,918)	81 (9.,075)
Total	93 (9,743)	122 (13,639)

( ) = Number of measured cell nuclei

Table 2. Cell and specimen classification results from severe dysplasia

Group	No. cases	Specimen classification		
		Progression	Nonprogression	Correct (%)
3 year				
Progression	24	18	6	75.0
Nonprogression	19	6	13	68.4
5 year				
Progression	14	12	2	85.7
Nonprogression	10	2	8	80.0
9 year				
Progression	7	6	1	85.7
Nonprogression	19	4	15	78.9
Total				
Progression	45	36	9	80.0
Nonprogression	48	12	36	75.0

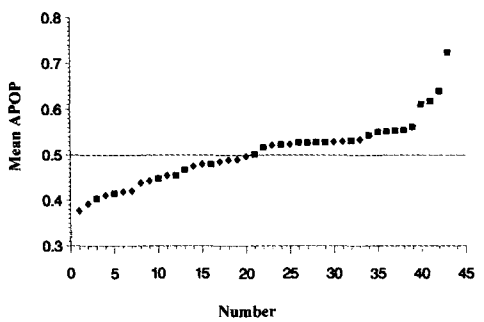

Fig. 1. Specimen classification results in patients with severe dysplasia with nonprogression within three years (◆) and progression within three years (■).

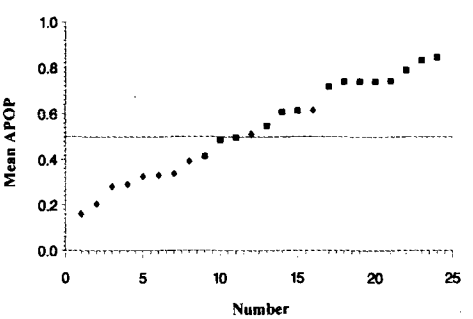

Fig. 2. Specimen classification results in patients with severe dysplasia with nonprogression within five years (◆) and progression within five years (■).

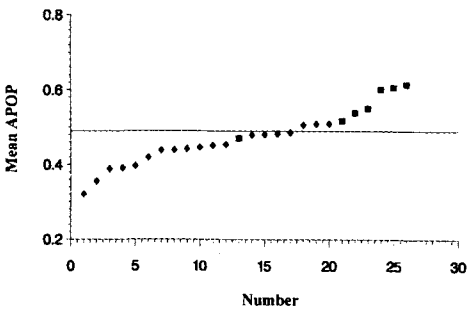

Fig. 3. Specimen classification results in patients with severe dysplasia with nonprogression within nine years (◆) and progression within nine years (■).

Results of 122 cytologically diagnosed mild dysplasia cases by image analysis is shown in Table 3. 16, 13 and 12 cases progressed to carcinoma in 3, 5 and 9 years, respectively. Correct cell classification rates were 93.8% (15/16) (P<0.0001), 76.9% (10/13) and 83.3% (10/12) (P<0.001), respectively. The other 81 cases remained stable or regressed to normal within 3, 5 and 9 years that were used as the control. The correct decisions were 88.9% (24/27), 70.4% (19/27) and 77.8% (21/27), respectively (Figures 4, 5, 6). NR1 (short runs emphasis of runlength distribution of flat texture), C06 (sum average of co-occurrence of extinction) and HUSPAN (range of upper skeleton) were the most important chromatin features to discriminate progression group within 3 years. Using the main chromatin features NC3 (correlation of co-occurrence of flat texture), NR1, C06 once again was discriminated progression group within 5 years. By means of the chromatin features MFRANG, MFM3 (skewness of local multifractal dimension) and C014 (local mean of co-occurrence of extinction) was discreminated progression group within 9 years. The data show that correct specimen classification rate of 85.4% could be achieved in cases of mild dysplasia using chromatin features (Table 3).

Table 3. Cell and specimen classification results from mild dysplasia

Group	No. cases	Specimen classification		
		Progression	Nonprogression	Correct (%)
3 year				
Progression	16	15	1	93.8
Nonprogression	27	3	24	88.9
5 year				
Progression	13	10	3	76.9
Nonprogression	27	8	19	70.4
9 year				
Progression	12	10	2	83.3
Nonprogression	27	6	21	77.8
Total				
Progression	41	35	6	85.4
Nonprogression	81	17	64	79.0

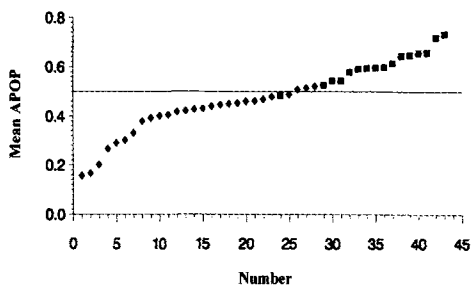

Fig. 4. Specimen classification results in patients with mild dysplasia with nonprogression within three years (◆) and progression within three years (■).

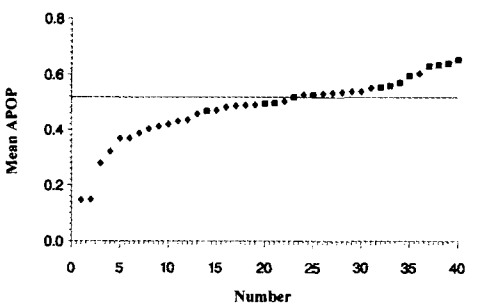

Fig. 5. Specimen classification results in patients with mild dysplasia with nonprogression within five years (◆) and progression within five years (■).

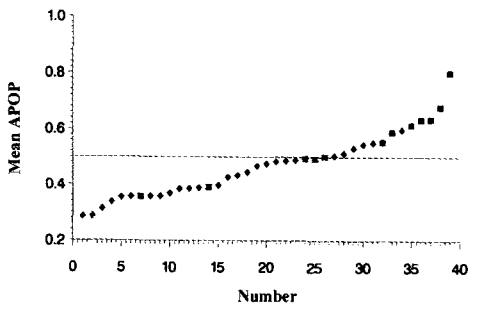

Fig. 6. Specimen classification results in patients with mild dysplasia with nonprogression within nine years (◆) and progression within nine years (■).

DISCUSSION

In 1966 Mendelsohn et al.[5] suggested that the recording of high resolution images and subsequent analysis by a digital computer could lead to the automated recognition and classification of five principal types of leukocytes. That classic paper applied new concepts to microscopic image analysis and statistical classifications. In 1980 and 1981 Burger[6] and Wied[7] noted such subvisual clues as marker features for neoplastic events in the uterine cervix. Subtle changes in normal-appearing epithelial cells adjacent to malignant tumors, or malignancy associated changes, can be detected by nuclear texture measurements. In 1997 Gao F et al.[8] obtained surprisingly good results from HRIA to discriminate severe esophageal dysplasia with progression and nonprogression. These preliminary results suggest that HRIA is a useful technique and highly sensitive for forecasting esophageal carcinoma. In this study, the progression to carcinoma within several years and nonprogression could be discriminated using texture features. Over 80% of severe dysplasia and over 85% of mild dysplasia can be correctly classified by means of benign-looking intermediate cells. We found that the behavior of both dysplasia types were similar: some dysplasia lesions are not true precancerous lesions but are nonspecific cell alterations. As it is well know that DNA is the basis of cell growth, differentiation and reproduction. The morphological changes such as an enlarged nucleus, hyperchromasia and the nuclear irregularity of the cells are the external appearance of DNA alteration. Therefore with chromatin features investigation, we may know the deeper significance and fundamental change in the cells. To date, efforts at early detection of esophageal cancer and precancerous lesions have concentrated on cytologic or histologic categorization. Nearly all dysplasia patients had their diagnoses made by cytologic and histologic criteria in clinical chemoprevention trials. It is not possible to obtain much information on the deeper layers of precancerous lesion cells by morphologic methods. This has proven difficult when depending on morphologic observation only since many cases have the same morphology, but the biologic behavior is quite different. Our results show that texture features can forecast precancerous lesions and can also be employed as surrogate end point biomarkers. HRIA, which is a powerful tool in cytologic diagnosis and research, can provide us with important information that is impossible to observe through routine methods. Cell measurement of texture features improves cytologic diagnosis and could be used to monitor progression of lesions as well as treatment.

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