Review and Progress of the Pathologic Research on Esophageal Carcinoma in Henan, China

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Abstract: In the first part of this paper, we briefly reviewed the history of pathologic research on esophageal carcinoma in Henan Province of China. In the research, the excellent work of Professor Qiong Shen, a famous pathologist, is prominently introduced, that includes cytologic diagnosis of esophageal carcinoma, classification of early esophageal carcinoma and the prevention of the esophageal carcinoma, etc. And then, the advance of pathologic research on esophageal carcinoma in Henan province is described as follows: 1. Morphometry research of esophageal carcinoma. 2. The relationship between Langerhans cells and esophageal carcinoma. 3. The relationship between apoptosis and esophageal carcinoma. 4. The related immunohistochemical markers of esophageal carcinoma. 5. The molecular biology and therapy research on esophageal carcinoma. [Life Science Journal. 2006;3(3):1−5] (ISSN: 1097−8135).

1 Introduction

Esophageal carcinoma is a kind of common malignant tumors which is seriously harmful to human being. In the world, it appears striking and puzzling differences in geographic incidence. Statistics from WHO (World Health Organization) show that the morbidity and mortality of esophageal carcinoma was the highest in China. The morbidity and mortality of esophageal carcinoma of Chinese male was 6.4/100,000, 31.66/100,000 and that of Chinese female was 20.0/100,000, 15.93/100,000. In 1997, 46.6% of the dead who died of esophageal carcinoma were Chinese. Meanwhile, in China, the highest morbidity and mortality of esophageal carcinoma was in Henan Province. In 1980, the mean mortality of esophageal carcinoma was 33.22/100.000, which was higher than that of any other areas in China.

In 1958, a preliminary survey on epidemiology of esophageal carcinoma was finished. It was found that the prevalence of esophageal carcinoma was much more severe in north of Henan Province than in other regions, especially in Linxian County (Linzhou City now). In 1959, under the guidance of China national leading pathologists, a doctor team from Henan Medical College worked in Linxian County and studied on etiology, pathogenesis, and cytologic diagnosis of esophageal carcinoma. Professors, such as Qiong Shen, Guiting Liu, and Songliang Qiu *et al*, were prominent members in the research group.

2 Cytologic Diagnosis and Pathologic Research on Early Esophageal Carcinoma

At the beginning of the research, autopsy was resisted by local people because of some of the obsolete traditional concepts. At that time, diagnosis of esophageal carcinoma mainly depended on X-ray barium meal visualization or esophagoscope, which was rough and made patients suffer a lot. Above all, most of the patients lost ideal chance for operation when they were found ill by the above two diagnostic methods. Placed in such a predicament, Professor Qiong Shen and his staff members decided to try cellular examination on esophageal carcinoma diagnosis. They invented the "Abrasive Cytological Balloon" and tried many times on themselves. After clinical test, the Balloon was proved to be effective and convenient for diagnosis of

esophageal carcinoma. From 1962, using the general survey with the Balloon in high-incidence areas, the diagnostic accuracy of advanced esophageal carcinoma had been 98.1%[1], and a lot of precancerous lesions of severe atypical hyperplasia and early esophageal carcinoma of asymptomatic patients were found. From the above data it was known that the Abrasive Cytological Balloon applied to the diagnosis of esophageal carcinoma[2,3]. At that time, the applications and disseminations of the Balloon gained high appraisal throughout the world.

Using the Balloon, a lot of asymptomatic or mild-symptomatic patients who suffered from esophageal carcinoma were found. The team with the leader of Professor Shen and Professor Qiu *et al* collected all the available materials of 362 cases of early esophageal carcinoma specimens and made the following conclusions: (1) Most minimal lesions were too small to be found. Only 10%-formalin-fixed lesions and iodine-smeared lesions could be found by naked eyes. (2) Peak morbidity of early esophageal carcinoma arose among people of 41-50 years old (51.1%), which was 6.3 years earlier than that of advanced esophageal carcinoma. (3) Based on the gross features, they, for the first time, distinguished esophageal carcinoma into 4 types: insidious type, erosion type, plaque type and papillary type, which was widely accepted and cited. (4) As for histological features, 35 insidious lesions were squamous cell carcinoma *in situ*, most erosion lesions were carcinoma *in situ* or were confined in mucosa, and more than half of plaque lesions invaded sub-mucosa. (5) Esophageal carcinoma always arose in more than one site, and it often progressed in the following way: normal mucosa → simple hyperplasia → atypical hyperplasia → carcinoma *in situ* →invasive carcinoma[4].

3　Etiology of Esophageal Carcinoma

3.1　Fungi

Much on-site inspection showed that people of Linxian County usually took mildewed and rotten food (such as pickle). Liu *et al*, for the first time, succeeded in inducing esophageal carcinoma on albino rats with natural rotten food, and made such conclusion that rotten food enhanced the carcinogenesis of nitrosamine[5]. He isolated the fungi from local grain and found that five kinds of fungi, including *Alternaria alternata*, had much higher contaminating possibility than that in low-incidence areas. It was known by animal studies that alternariol monomethyl ether (AME) and alternariol (AOH) were active components of *Alternaria alternata*, and both of them enhanced hyperplasia of fetal esophageal epithelia *in vitro* and even cancerization[6].

3.2　Virus

With electron microscope, for the first time, Hu found virus-like particles in the cytoplasm of esophageal carcinoma cells[7]. Then, with *in situ* hybridization, Chang *et al* detected DNA of HPV6, 11, 16, 18 within precancerous lesions and cancer tissues, which indicated that infection of HPV might be concerned with development of esophageal carcinoma[8].

4　Prevention of the Cancerization among High-risk Group

As we know that epithelia hyperplasia was the only way to cancerization. Based on this theory, using rough riboflavin and rabdosia rubesens, Professor Shen *et al* began the research of preventing cancerization. From 1988 to 1992, the results proved that long-term taking rough riboflavin could prevent 57.1% of severe atypical hyperplasia from cancerization, which indicated that rough riboflavin had obvious function of preventing cancerization[9].

5　Morphometry Research of Esophageal Carcinoma

At the first time of esophageal cellular research, microscope micrometer was used to measure the nuclear dimension of hyperplasia cells and made quantification to different hyperplasia grades. In 1990, Professor Zhang *et al* started a new method. Using computer image texture analysis and correlation grid methods, different texture features of normal mucosa, atypical hyperplasia epithelia and carcinoma *in situ* of human esophagus were observed. The texture measures and the data of correlation grid test showed significant difference between severe atypical hyperplasia epithelia and carcinoma *in situ*. The computer image texture analysis might correctly distinguish atypical hyperplasia in esophageal precancerous change from carcinoma *in situ*. With double-blind detection, the accuracy of this technique reached above 90%. This method might have affirmative practical value in the early diagnosis of esophageal carcinoma[10].

6　Relationship between Langerhans Cells and Esophageal Carcinoma

Langerhans cells (LC) were members of dendritic cells (DC). They were successively found in epidermis and other squamous cells covered mucosa, such as oral cavity, pharynx, larynx, rectum, cervix and vagina. They inlayed among ker-

atinocytes and were not able to be found with HE stain. While, with ATPase cellular chemistry or immunohistochemistry of S-100 and OKT6, their morphous could be clearly observed. The main function of them was to present antigens to T lymphocytes. There were Fc-receptors, C3b and immune associated antigen (Ia antigen) on the surface of LC. LC could be classified into 6 types according to their different dendrites.

From 1990, Zhang took the lead in observing the morphous, distribution, and quantity of LC with ATPase (+), S-100 (+), OKT6 (+) in esophageal lesions. Conclusions were made as follows: the expression of S-100 and OKT6 decreased as the lesion progressed, least ATPase positive LC were found in severe atypical hyperplasia epithelia, and most were found in carcinoma *in situ*. Most LC in normal mucosa had less but long and obvious dendrites. While the dendrites became more but shorter in severe atypical hyperplasia epithelia and in carcinoma. LC mainly distributed in the lower layers of normal epithelia, but appeared anywhere in carcinoma *in situ*. At the same time, LC were found to be close to T-lymphocytes and cancer cells. All of the above indicated that different subtypes of LC took part in the progress of esophageal carcinoma[11].

Combining the observation of HPV infection and LC' change, it was found that HPV infection decreased the quantity of LC, which might cooperate with other carcinogens and worked in the progress of esophageal carcinoma[12].

7 Relationship between Apoptosis and Esophageal Carcinoma

With TUNEL technique and immunohistochemistry, apoptosis was found to be related to differentiation of esophageal carcinoma. Change of ICE protein could be an indicator of early cancerization. Cisplatin could induce apoptosis of Eca-109 cells, and DNA degradation was the important change during apoptosis[13].

8 Immunohistochemical Markers of Esophageal Carcinoma

More than 20 targets had been involved in this research and 4 of them would be briefly mentioned.

8.1 P53 protein

The research indicated that 60.0% of carcinoma presented P53 protein stain positive, while 42.9% − 66.7% of atypical hyperplasia epithelia and carcinoma *in situ* presented P53 protein stain positive. The positive rate of P53 protein staining was related to the differentiation, infiltration and metastasis of esophageal carcinoma[14].

8.2 P16 protein

The positive rate of P16 protein staining decreased in the order of normal mucosa, atypical hyperplasia epithelia and cancer tissue, and it decreased as differentiation became poorer[15].

8.3 nm23-H1

In the adjacent non-cancerous mucosa, the positive rate of nm23-H1 staining decreased as the lesion became poorer; in the cancerous tissue, the poorer the lesion was, the lower the positive rate of nm23-H1 staining was; and it was the lowest in lymph node metastasis cases[16].

8.4 GST-π

The positive rate of GST-π staining was relatively high in normal and simple hyperplasial epithelia, while it decreased in atypical hyperplasia epithelia and cancer tissues. The result indicated that GST-π was early enzymologic change of esophageal carcinoma[17].

9 Molecular Biology and Therapy on Esophageal Carcinoma

9.1 p53 gene

With PCR-SSCP silver staining, mutation of exon 5, 6, 7, 8 of p53 gene could be observed. It was found that 32.5% cases presented p53 gene mutation, and the mutation rate of lymph node metastasis group was obviously higher than that of non-metastasis group, suggesting that mutation of p53 gene might contribute to the development of esophageal carcinoma.

9.2 p16 gene

Through observing the mutation of p16 gene in adjacent non-cancerous mucosa and in cancer tissue, it was found that the mutation rate was 27.5% in cancerous mucosa and no mutation occurred in adjacent non-cancerous mucosa. Meanwhile, the mutation rate decreased as differentiation became poorer and it increased as adventitia infiltration and lymph node metastasis happened[18].

9.3 GSTs isoenzyme gene

Through RNA dot blot hybridization, it was found that transcriptional level of GST gene was higher in cancer tissue. GST-π was active form of GSTs isoenzyme in esophageal carcinoma. Altofrequency of positive GST-π might indicate its important role in the process of esophageal carcinoma.

9.4 Telomerase activity and targeted therapy of esophageal carcinoma by antisense oligodeoxynucleotide

Applying TRAP-ELASA quantitative analysis and TRAP silver staining, telomerase activity was respectively detected in cancer tissues, atypical

hyperplasia tissues and normal esophageal mucosa. With *in situ* hybridization, expression of catalytic subunit hTR mRNA of telomerase was detected. With Southern blot and chemiluminescence methods, the length of the telomere was measured. 5 synthetic ASODN with different blocked gene locus were respectively transfected into esophageal cancer cells, and then the cells were subcutaneously planted to nude mice. By this way, the apoptosis induced by ASODN and its inhibitory effect on cell proliferation could be directly observed. All results indicated that activation of telomerase was an early event of tumorigenesis in esophagus and ASODN-t3 could induce apoptosis of tumor cells through inhibiting telomerase activity[19].

9.5 DNA polymerase β gene (polβ)

Professor Dong was the first to do systemic study on mutation of polβ in esophageal carcinoma[20]. With RT-PCR, SSCP and sequence analysis, polβ was studied in cancer tissues and adjacent non-cancerous mucosa. It could be seen that mutation rate of polβ was as high as 44% in cancer tissues, while it was only 4% in adjacent non-cancerous mucosa. So such conclusion could be made that mutation of polβ was related to progression of esophageal carcinoma.

9.6 Screening and identification of esophageal cancer associated gene

With mRNA differential display and suppression subtractive hybridization (SSH), three esophageal cancer associated genes 3y59, c57 and ECAG1 were found in specimens from high-incidence areas. Through homology search in GenBank, no identical genes were found. They all had been registered in GenBank. The work is going on[21].

9.7 Relationship between heparanase and infiltration, metastasis of esophageal carcinoma

It was proved that heparanase was able to destroy extracellular matrix (ECM) and basement membrane (BM) and played some roles in cancerous angiogenesis, infiltration and metastasis. But the definite relationship and mechanism was unclear. To find the answer, with the guidance of Professor Zhang, the following studies had recently been finished:

A. 54 cases of specimens were obtained from high-incidence areas. With *in situ* hybridization and RT-PCR, expression of heparanase was detected respectively in cancer tissues, atypical hyperplasia of adjacent non-cancerous mucosa and normal mucosa of the above 54 specimens. It was found that the protein and mRNA expression of heparanse was related not only to metastasis but also to infil-

tration depth of esophageal carcinoma[22,23].

B. With RT-PCR, the expression of heparanse in peripheral blood lymphocytes of patients with esophageal cancer was detected. The study suggested that the level of hepatanase expression was higher in group with metastasis than that in group without metastasis. The result indicated that expression of heparanse in peripheral blood lymphocytes, to some extent, could reflect the metastatic state of esophageal carcinoma[24].

C. Using antiesense oligodeoxynucleotide technique, ASODN of different concentration were transfected into EC9706, and then the expression of protein and mRNA of heparanase was detected. It was found that heparanase ASODN could weaken the infiltrative and metastatic ability of cancer cells by inhibiting the expression of heparanase[25].

D. Expression of heparanase in nude mice transplanted tumor indicated that hepatanase ASOND could inhibit the expression of heparanase *in vivo*, which confirmed the above conclusion.

9.8 Expression of differentiation-related gene NDRG Ⅰ and differentiation-induced therapy

Discovered in 1997, NDRG Ⅰ (N-myc downstream regulated gene Ⅰ) was a kind of gene which was related to differentiation. It always presented low expression in many tumors. Until now, no article reported about NDRG Ⅰ expression in esophageal carcinoma. Recently, using molecular biological tech and IHC, we studied on expression of NDRG Ⅰ mRNA and protein in normal esophageal mucosa, atypical hyperplasia and cancer tissues. Differentiation-induced therapy was involved, too. The study indicated that phorbol ester, ratinoic acid, Vit D_3 and sodium butyrate could induce the expression of NDRG Ⅰ in esophageal cancer cells, which showed us a new clue for gene therapy of esophageal carcinoma[26,27].

Based on hard work of more than 40 years, we have made some progresses in the field of esophageal carcinoma. At present, we have built Henan Key Laboratory of Tumor Pathology, and a lot of young doctors have been trained to be eligible researchers. Although there still are a lot of problems to deal with in this field, we are deeply convinced that we will have a bright future.

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