Scientific Research and Essays Vol. 7(2), pp. 165-169, 16 January, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.1106
ISSN 1992-2248 ©2012 Academic Journals

Full Length Research Paper

The alterations of tyrosine and tryptophane residues along with the evolution of tumor: Determination by synchronous fluorescence spectra

Ming Han[1], Zhexuan Lin[2], Yuan Zhang[2]*, Lin Peng[3] and Yaowen Chen[4]

[1]Emergency Department of Second Affiliated Hospital, Shantou University Medical College, Shantou, China.
[2]Central Laboratory, Shantou University Medical College, Xinling Road 22, Shantou, Guangdong 515041, China.
[3]Clinical Laboratory of Affiliated Tumor Hospital, Shantou University Medical College, Shantou, China.
[4]Central Laboratory, Shantou University, Shantou, China.

Accepted 23 December, 2011

Synchronous fluorescence spectra were applied to assess tyrosine (Tyr) and tryptophane (Trp) residues in the plasma of patients with hepatocellular carcinoma, tumor-bearing mice and in cultured cells (HepG2 and L-02). The results showed for the first time that the fluorescent intensities of Tyr and Trp residues increased significantly in the plasma proteins of patients suffering hepatocellular carcinoma. There is a correlation between the increase of murine plasma Tyr or Trp residues fluorescent intensities and the increasing time of tumor-bearing, indicating that the alterations of Tyr and Trp residues may be associated with tumor development. On the contrary, the fluorescent intensities of Tyr and Trp residues in tumor tissue or HepG2 cells decreased along with the increasing time of tumor-bearing or culture. These results suggested a profound imbalance of protein metabolism in tumor evolution process.

Key words: Synchronous fluorescence spectra, tyrosine residue, tryptophane residue, hepatocellular carcinoma.

INTRODUCTION

Diagnostic techniques based on fluorescence spectroscopy have been developed in the last decade (Manoharan et al., 1998; Wagnières et al., 1998). They have the potential to link the biochemical and morphologic properties of tissues, and represent an important step toward advances in diagnostic and therapeutic medical applications. One of the most widely explored application of fluorescence spectroscopy is autofluorescence which is the fluorescence obtained from tissue fluorophores and can be used to obtain diagnositc information in carcinoma (Meng et al., 2007; Sheng et al., 2001; D'Hallewin et al., 2002). However, the alterations of fluorescence intensities for aromatic amino acid residues in proteins have not been reported in neoplasma diseases. The major fluorophores contributing to endogenous fluorescence of proteins are tyrosine (Tyr) and tryptophane (Trp) residues (Grigoryan et al., 2008). In ordinary fluorescence spectra, the excitation wave lengths of Tyr and Trp residues were 275 and 295 nm respectively, with overlapping emission peak, making it impossible to be distinguished.

Synchronous fluorescence method is a technique wherein simultaneous scan of both the excitation and emission spectra are done to maintain a constant difference between the emission and excitation wavelengths, $\Delta\lambda = \lambda em - \lambda ex$. This method has been used for scanning ranges of $\Delta\lambda \leq 20$ nm or $\Delta\lambda \geq 60$ nm to observe fluorescence of Tyr and Trp residues successfully (Grigoryan et al., 2008; Ma et al., 1999; Du et al., 2001). Previous studies have demonstrated that the contents of free Tyr and Trp were in great imbalance in tumor patients (Wu and Bauer, 1960; Yan et al., 2007). We addressed the question whether their residues in proteins were also involved in tumor evolution process.

To this end, synchronous fluorescence method was applied to determine the fluorescent intensities of Tyr and

*Corresponding author. E-mail: yzhang@stu.edu.cn.

Trp residues in the plasma and cell lysates, and to explore their physiopathologic implications.

MATERIALS AND METHODS

Phenylmethylsulfonyl fluoride (PMSF) was purchased from Sangon Biological (Shanghai, China). Dulbecco's Modified Eagle Media/Nutrient Mixture F-12 [DMEM/F12 (v/v, 1:1)] and new born calf serum (NCS) were from Invitrogen (USA). All other chemicals in this study were of analytical grade.

Cell lines

Human hepatoma (HepG2) cell line and human hepatocytes (L-02) were gifted from Department of Immunology, Shantou University Medical College. Mouse hepatocarcinoma cells (H22) were obtained from Shantou University, College of Science.

Animals

The BALB/c mice and C57BL/6 mice (weight 22 ~ 25 g) were obtained from Sino British SIPPR/BK Lab Animal Ltd. (Shanghai), and placed in cages and housed in a temperature-controlled room with a 12 h light, 12 h dark cycle. All the research was conducted in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and was approved by the Medical Animal Care and Welfare Committee of Shantou University Medical College.

Culture of cells

HepG2 cells and L-02 cells were cultured in DMEM/F12 (1:1, v/v) with 10% NCS for various duration of time (6, 12, 24 and 48 h). At the end of the incubation, the culture media were removed and the cells were rinsed with PBS 3 times before harvest. Then, the cells were lysed in ice-cold 0.01 M PBS with 1 mM PMSF and vortexed for 10 s followed by sonication for 2 min, and insoluble material was removed by centrifugation at 15,000 g for 30 min. The supernatants were used for synchronous fluorescence measurement and protein content determination.

Animal tumor model

H22 cells were transferred in abdomen cavity of BALB/c mouse. The ascites were taken from the mouse and diluted with saline (0.9%) to 1×10^7/ml. A total of 200 µl of 1×10^7/ml H22 cells were inoculated subcutaneously at the right armpit of C57BL/6 mouse to establish a tumor model (Wang et al., 2000), same volume of vehicle was inoculated to C57BL/6 mouse as control. Animals were sacrificed at different time points (2, 4, 6, 9, 12 and 14 d) after inoculation. Blood samples were collected by cardiac puncture and centrifuged. The resulting plasma was obtained for determination of synchronous fluorescence. The neoplasm tissue was also collected and homogenized in 0.01 M PBS with 1 mM PMSF, then submitted to centrifugation at 15,000 g for 30 min. The supernatants were collected for synchronous fluorescence measurement and protein content determination.

Blood collection from patients and healthy volunteers

Five patients with hepatocellular carcinoma admitted to Affiliated Tumor hospital (Medical College of Shantou University) and 4 healthy volunteers were enrolled in the study. Peripheral venous blood samples were obtained from patients at admission; plasma was collected and stored at -70°C until assay.

Synchronous-scan spectrofluorometry

Synchronous-scan spectrofluorometry was performed with a spectrofluorometer (RF-5000) from Shimadzu Corporation. For synchronous excitation measurements, both excitation and emission monochromators were locked together and scanned simultaneously with a constant wavelength difference $\Delta\lambda = \lambda_{em} - \lambda_{ex}$. The synchronous fluorescence spectra were obtained by scanning simultaneously at constant-wavelength difference of $\Delta\lambda$ = 20 nm for Tyr residues, and $\Delta\lambda$ = 80 nm for Trp residues, respectively. The synchronous fluorescence intensities were measured at the synchronous maxima of each residues (for Tyr λ_{max} = 318 nm, for Trp λ_{max} = 350 nm, respectively). The excitation and emission slit widths were set at 3 nm each. The results were expressed as fluorescent intensity/mg proteins. Protein concentration was determined by BCA detection kit.

Statistical analysis

All data in this study are presented as mean ± SD. Comparison between groups was performed with the unpaired t-test. All these statistical analyses were carried out using prism 5. P values less than 0.05 were accepted as statistically significant.

RESULTS

Synchronous-scan spectroscopy in aqueous suspension

In ordinary fluorescence spectra, the maximum excitation wavelengths of Tyr and Trp were 275 and 295 nm respectively, but the maximum emission wavelengths of them overlapped at 350 nm. The synchronous fluorescence peaks of Tyr and Trp residues are located at 318 and 350 nm when Δλ = 20 nm and Δλ = 80 nm respectively, and did not shift regardless of the sample variations. Therefore, for Δλ = 20 nm, λem = 318 nm was recorded, and the fluorescence signal belongs to the Tyr residues, while for Δλ = 80 nm, λem = 350 nm was recorded and the fluorescence signal belongs to the Trp residues.

The fluorescent intensities of Tyr and Trp residues in cultured cells

As shown in Table 1, with the increasing time of culture, fluorescent intensities of Tyr and Trp residues decreased in HepG2 hepatoma cells lysates. In L-02 hepatocyte lysates, relatively lower fluorescent intensities were observed within 6 h of culture and the fluorescent intensities were peaked at 12 h followed by the decrease of intensities after 24 h culture. The fluorescent intensities of Tyr and Trp residues in HepG2 lysates were higher

Table 1. The fluorescent intensities of Tyr and Trp residues in HepG2 and L-02 cells (fluorescent intensity/mg proteins).

Time (h)	Tyr		Trp	
	L-02	HepG2	L-02	HepG2
6	778.3 ± 97.4	1068.4 ± 57.7*	2536.5 ± 359.0	3546.2 ± 108.2*
12	1017.0 ± 205.5	709.4 ± 94.6*	3184 ± 788.1	2285.2 ± 307.6*
24	800.7 ± 57.5	439.0 ± 31.1*	2520 ± 191.4	1325 ± 106.3*
48	460.3 ± 34.4	346.2 ± 14.9	1374.3 ± 121.8	958.3 ± 39.1

* P<0.05 versus L-02 at the same time.

than those in L-02 lysates at 6 h culture (P<0.05), whereas the fluorescent intensities of Tyr and Trp residues in HepG2 lysates were lower than those in L-02 lystaes at both 12 and 24 h time point (P<0.05).

The fluorescent intensities of Tyr and Trp residues in mice plasma and neoplastic tissue

4 days after H22 inoculation, neoplastic tissue could be observed at the right armpit of mouse and grew rapidly in the following days. Synchronous fluorescence spectra showed that the fluorescent intensities of Tyr and Trp residues in neoplastic tissue decreased along with the increasing of inoculation time (P<0.0001) (Figure 1A). However, the fluorescent intensities of Tyr and Trp residues in plasma increased with the increasing time of inoculation (P<0.0001) (Figure 1B).

The fluorescent intensities of Tyr and Trp residues in the plasma of patients with hepatocellular carcinoma

As shown in Figure 2, fluorescent intensities of Tyr and Trp residues were higher in the plasma of tumor patients than those of healthy controls (P<0.05).

DISCUSSION

In the present study, synchronous fluorescence spectra were used for the first time to determine the variations of Tyr and Trp residues levels in malignant tumor. The results showed that the fluorescent intensities of Tyr and Trp residues were higher in the plasma of tumor patients than those of normal controls. This was consistent with the results of animal experiments which showed higher fluorescent intensities of Tyr and Trp residues in the plasma of tumor-bearing mice than those of control. These data suggested that Tyr-rich or Trp rich proteins might decrease in the plasma of tumor-bearing mice and patients. Furthermore, we found that the fluorescent intensities of Tyr and Trp residues in the plasma or neoplastic tissue showed a time-dependent variation after H22 cells inoculation, indicating that the changes of Tyr-rich or Trp-rich proteins may be correlated with tumor development. It has been reported that the metabolism of protein and amino acids was in great imbalance in tumor patients (Wang et al., 2001; LüF et al., 2004). Because of the difficulty in determination of amino acid residues, previous studies focused on the free amino acids in plasma, and showed that aromatic amino acids - Tyr and Trp- increased in plasma of the patients suffering malignant diseases (Hagmüller et al., 1995; Nishizaki et al., 1995; Jing et al., 2001).

Amino acids are the basis of protein synthesis. Therefore, the amino acid imbalance may disturb protein synthesis and result in the changes of protein categories. This can explain our results that Tyr and Trp residues level changed in the plasma of tumor patients or tumor bearing mice. In addition, the present study showed a decrease of fluorescent intensities of Tyr and Trp residues in neoplastic tissue along with the increasing time of tumor-bearing, which was in contrast to what have been observed in the plasma. This result is also consistent with previous studies showing that the contents of free Tyr and Trp in gastric and colonic carcinoma tissue were significantly increased compared with those of normal mucosa (Wu and Bauer, 1960; Yan et al., 2007). Current study also demonstrated a decrease of fluorescent intensities of Tyr and Trp residues in hepatoma cells along with duration of culture and the lower fluorescent intensities than those of normal hepatocyte line. Similar studies showed that breast cancer cells had a lower free Tyr content than that of normal epithelial cell line (Kao et al., 2010). There may be two reasons to explain these results. Firstly, cancer cells may reduce the uptake of aromatic acids when cells canceration as reported that free Tyr and Trp were lower in cancer cells (Kao et al., 2010). Thus, the deficient resources would result in the reduction of Tyr-rich or Trp-rich proteins synthesis. Secondly, the capacity of Tyr-rich or Trp-rich proteins catabolism increased.

Previous study has described an increase of free Trp in culture medium of cancer cells which may be resulted from the catabolic rate increase (Hagmüller et al., 1995).

Aromatic amino acids residues were recently reported to be involved in nucleic acid recognition (Baker et al.,

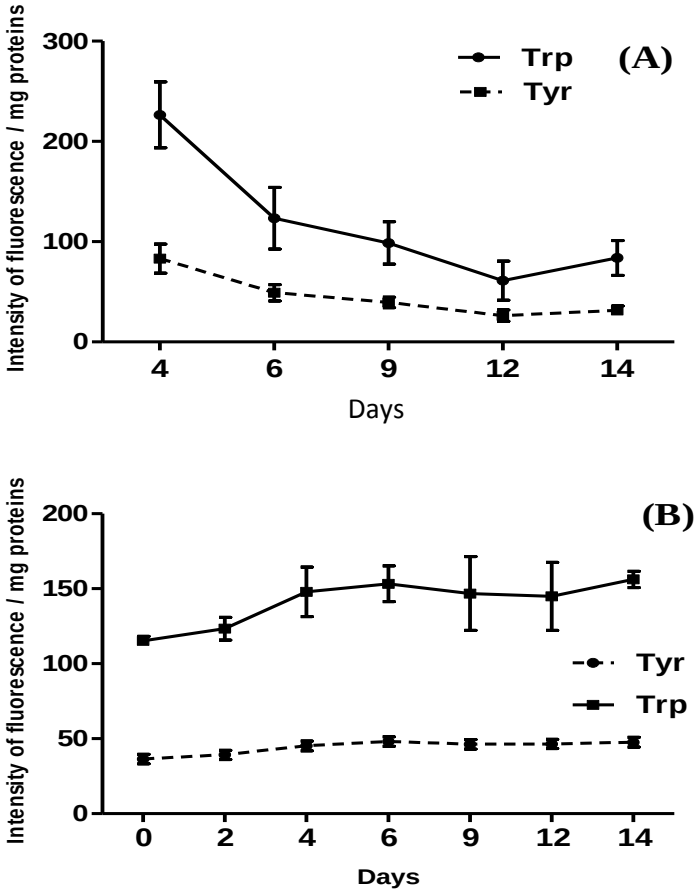

Figure 1. The fluorescent intensities of Tyr and Trp residues in mice neoplastic tissue and plasma. The fluorescent intensities of Tyr and Trp residues in neoplastic tissue decreased along with the increasing of inoculation time (P<0.0001) (A) and the fluorescent intensities of Tyr and Trp residues in plasma increased with the increasing time of inoculation (P<0.0001) (B).

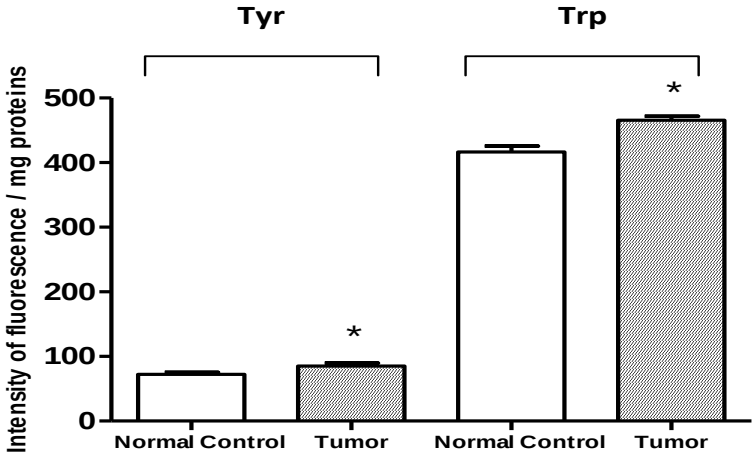

Figure 2. The fluorescent intensities of Tyr and Trp residues in the plasma of hepatocellular carcinoma patients and healthy controls. Fluorescent intensities of Tyr and Trp residues were higher in the plasma of tumor patients than those of healthy controls. * P<0.05 versus normal control.

2007). Thus, the variations of Tyr or Trp residues may contribute to the evolution of tumor-correlated gene expression, and play a role in tumor evolution and process. In conclusion, our study demonstrated that Synchronous fluorescence spectra could be used to determine Tyr and Trp residues in proteins. To the best of our knowledge, this is the first study showing that Tyr and Trp residues were increased in the plasma proteins of tumor bearing patients and mice which may be correlated with tumor development. On the contrary, Tyr and Trp residues in proteins of tumor tissue or cancer cells were decreased. It remained unknown whether these changes acted as a biomarker for protein metabolism imbalance in malignant diseases or they contributed to the tumor evolution. It is worthy of further investigation.

ACKNOWLEDGEMENTS

This work is supported by the National Natural Science Foundation of China (project number 81172088 and 60971075), Science and Technology Planning Project of Shantou City (2008-64) and Shantou University Scientific Research Foundation for the Doctoral Scholar (2010).

REFERENCES

Baker CM, Grant GH (2007). Role of aromatic amino acids in protein-nucleic acid recognition. Biopolymers, 85: 456.

D'Hallewin MA, Bezdetnaya L , Guillemin F (2002). Fluorescence detection of bladder cancer: a review. Eur Urol., 42: 417.

Du XL, Li RC, Wang K (2001). A study of the function of terbium(Ⅲ) and apotransferrin by Synchronous fluorescence spectra. Chinese Sci. Bull., 46: 394.

Grigoryan KR, Aznauryan MG, Bagramyan NA, Gevorkyan LG, Markaryan ShA (2008). Spectroscopic determination of binding between human serum albumin and a platinum(II) dimethylsulfoxide complex. J. Appl. Spectroscopy, 75: 593.

Hagmüller E, Kollmar HB, Günther HJ, Holm E, Trede M (1995). Protein metabolism in human colon carcinomas: in vivo investigations using a modified tracer technique with L-[1-13C]leucine. Cancer Res., 55: 1160.

Jing Y, Huang MY, Dai Y, Xu Y (2001). The relationship between cencer and free amino acid. Amino Acids Biotic Resour., 23: 51.

Kao YY, Liu KT, Huang MF, Chiu TC, Chang HT (2010). Analysis of amino acids and biogenic amines in breast cancer cells by capillary electrophoresis using polymer solutions containing sodium dodecyl sulfate. J. Chromatogr. A, 1217: 582.

Lü F, Qin ZY, Yang WB, Qi YX, Li YM (2004). A DNA vaccine against extracellular domains 1-3 of flk-1 and its immune preventive and therapeutic effects against H22 tumor cell in vivo. World J. Gastroenterol.,10: 2039.

Ma CQ, Li KA, Zhao FL, Tong SY, Azurol S (1999). A Study on the Reaction Mechanism between Chrome- and Bovine Serum Albumin. Acta Chimica Sinica, 57: 389.

Manoharan R, Shafer K, Perelman L, Chen K, Deinum G, Fitzmaurice M, Myles J, Crowe J, Dasari RR, Feld MS (1998). Raman spectroscopy and fluorescence photon migration for breast cancer diagnosis and imaging. Photochem Photobiol., 67: 15.

Meng T, Xu DF, Xu YZ, Zhao Y, Wang DJ, Wu JG (2007). Investigation on the fluorescence spectroscopy of normal and malignant rectum tissues. Guang Pu Xue Yu Guang Pu Fen Xi, 27: 1156.

Nishizaki T, Matsumata T, Taketomi A, Yamamoto K, Sugimachi K (1995). Levels of amino acids in human hepatocellular carcinoma and adjacent liver tissue. Nutr. Cancer, 23: 85.

Sheng JQ, Li SR, Gao G, Li SY, Wang LP (2001). Feature of LIAF spectra for detection of colorectal carcinoma and its mechanism. Chinese J. Laser Med. Surg., 10: 239-242.

Wagnières GA, Star WM, Wilson BC (1998). In vivo fluorescence spectroscopy and imaging for oncological applications. Photochem Photobiol., 68:603.

Wang L, Li BY, Li QR (2000). The study on interrelation between gastric cancer tissue free amino acid and tumor volume. Parenteral Enteral Nutr., 7: 41.

Wang L, Tong XQ, Li QR(2001). The study on interrelation between colonic carcinoma tissue free amino acid and tumor volume. Parenteral Enteral Nutr., 8: 221.

Wu C, Bauer JM (1960). A study of free amino acids and of glutamine synthesis in tumor-bearing rats. Cancer Res., 20: 48.

Yan J, Lu YJ, Tan HR (2007). Research on the spectrum of free amino acid in serum and urine of patients with lung cancer. J. Clin. Pulm. Med., 12: 429.