World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i14.4210

World J Gastroenterol 2015 April 14; 21(14): 4210-4215
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Multiphoton microscopy for tumor regression grading after neoadjuvant treatment for colorectal carcinoma

Lian-Huang Li, Zhi-Fen Chen, Xing-Fu Wang, Shuang-Mu Zhuo, Hong-Sheng Li, Wei-Zhong Jiang, Guo-Xian Guan, Jian-Xin Chen

Lian-Huang Li, Shuang-Mu Zhuo, Hong-Sheng Li, Jian-Xin Chen, Institute of Laser and Optoelectronics Technology, Fujian Provincial Key Laboratory for Photonics Technology, Key Laboratory of Optoelectronic Science and Technology for Medicine of Ministry of Education, Fujian Normal University, Fuzhou 350007, Fujian Province, China
Zhi-Fen Chen, Wei-Zhong Jiang, Guo-Xian Guan, Department of Colorectal Surgery, Fujian Medical University Union Hospital, Fuzhou 350001, Fujian Province, China
Xing-Fu Wang, Department of Pathology, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350001, Fujian Province, China
Jian-Xin Chen, College of Photonic and Electronic Engineering, Fujian Normal University, Fuzhou 350007, Fujian Province, China
Author contributions: Li LH, Chen ZF and Wang XF contributed equally to this work; Li LH and Chen JX were responsible for study conception and design; Li LH and Li HS obtained the data; Chen ZF, Wang XF, Zhuo SM, Jiang WZ and Guan GX provided technical support; all authors contributed to analysis and interpretation of data, wrote, reviewed and approved the final manuscript.
Supported by Program for Changjiang Scholars and Innovative Research Team in University, No. IRT1115; the National Natural Science Foundation of China, No. 81271620; the Natural Science Foundation for Distinguished Young Scholars of Fujian Province, No. 2014J06016; the Youth Scientific Research Foundation of Fujian Provincial Department of Health (2013-2-36); and National Clinical Key Specialty Construction Project (General Surgery).
Ethics approval: This study was reviewed and approved by the Fujian Medical University Union Hospital Institutional Review Board.
Informed consent: All study participants provided informed written consent prior to study enrollment.
Conflict-of-interest: The authors declare that they have no conflict of interest.
Data sharing: No additional data are available.

Correspondence to: Jian-Xin Chen, PhD, Professor, College of Photonic and Electronic Engineering, Fujian Normal University, No. 8 Shangsan Road, Cangshan District, Fuzhou 350007, Fujian Province, China. chenjianxin@fjnu.edu.cn
Telephone: +86-591-22686078
Fax: +86-591-83465373
Received: November 4, 2014
Peer-review started: November 5, 2014
First decision: November 26, 2014
Revised: January 10, 2015
Accepted: January 21, 2015
Article in press: January 21, 2015
Published online: April 14, 2015

Abstract

AIM: To evaluate the feasibility of using multiphoton microscopy (MPM) to assess a tumor regression grading (TRG) system.

METHODS: Fresh specimens from seven patients with colorectal carcinoma undergoing neoadjuvant radiochemotherapy at the Fujian Medical University Union Hospital were obtained immediately after proctectomy. Specimens were serially sectioned (10 μm thickness) and used for MPM or stained with hematoxylin and eosin for comparison. Sections were imaged by MPM using 810 nm excitation, and images were collected in two wavelength channels corresponding to second-harmonic generation (SHG) and two-photon excited fluorescence (TPEF) signals. The ratio of these signal intensities was used to distinguish fibrosis from normal mucosal and serosal tissues.

RESULTS: TRG of specimens assessed by MPM

were in complete agreement with histologic grading performed by a consulting pathologist. SHG and TPEF images clearly revealed collagen fibers and fragmented elastic fibers in the muscularis propria specimens following neoadjuvant radiochemotherapy. Additionally, blood vessel hyperplasia was observed as thickening and fibrosis of the intima and media, which was accompanied by minimal inflammatory cell infiltration. Furthermore, the SHG/TPEF ratio in stromal fibrosis (4.15 ± 0.58) was significantly higher than those in the normal submucosal (2.31 ± 0.52) and serosal (1.47 ± 0.10) tissues (P < 0.001 for both). Analysis of emission spectra from cancerous tumor cells revealed two peaks corresponding to nicotinamide adenine dinucleotide hydrogen and flavin adenine dinucleotide signals; the ratio of these values was 1.19 ± 0.02, which is close to a normal metabolic state.

CONCLUSION: MPM can be used to perform real-time diagnosis of tumor response after neoadjuvant treatment, and can be applied to evaluate TRG.

Key words: Multiphoton microscopy; Neoadjuvant treatment; Second-harmonic generation; Tumor regression grading; Two-photon excited fluorescence

Core tip: This study evaluated the feasibility of using multiphoton microscopy for the assessment of a tumor regression grading system. Multiphoton microscopy allows diagnostic features of colorectal carcinoma treated with neoadjuvant therapy to be visualized. Quantitative image analyses can be used to distinguish fibrotic tissue from normal submucosal and serosal tissues. This is the first study demonstrating the application of multiphoton microscopy for tumor regression grading.

Li LH, Chen ZF, Wang XF, Zhuo SM, Li HS, Jiang WZ, Guan GX, Chen JX. Multiphoton microscopy for tumor regression grading after neoadjuvant treatment for colorectal carcinoma. *World J Gastroenterol* 2015; 21(14): 4210-4215 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i14/4210.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i14.4210

INTRODUCTION

For patients with locally advanced gastrointestinal cancer, neoadjuvant therapy followed by surgery or perioperative treatment provides a survival benefit over surgery alone, particularly for patients with complete or subtotal tumor regression[1-5]. As such, assessment of therapeutic response and evaluation of residual disease are very important. The response to therapy can be evaluated histologically *via* a tumor regression grading (TRG) system, which has been shown to correlate with survival[6,7]. The TRG system aims to categorize the extent of regressive changes with consideration of the percentage of the tumor that is residual and the degree of therapy-induced fibrosis[7-10]. This system provides valuable prognostic information, and may serve as a morphologic indicator for neoadjuvant treatment and surgery[11,12].

Histopathologic evaluation of resected specimens can be subject to crush artifacts and sampling error, and involves time-consuming pathologic procedures[13,14]. In contrast, multiphoton microscopy (MPM), which relies on the nonlinear optical processes of second-harmonic generation (SHG) and two-photon excited fluorescence (TPEF), provides high resolution visualization of cell morphology and tissue architecture without the use of exogenous contrast agents[15,16]. The value of this method for use in the TRG system has not been examined. Therefore, the purpose of this study was to evaluate the accuracy and feasibility of MPM for optical diagnoses with the TRG system.

MATERIALS AND METHODS

Specimen preparation

Fresh specimens were obtained immediately after proctectomy from seven patients with colorectal carcinoma undergoing neoadjuvant radiochemotherapy at the Fujian Medical University Union Hospital. Normal tissue specimens were also obtained 6 cm away from the cancer margin. This investigation was approved by the Institutional Review Board of the Fujian Medical University Union Hospital. Written informed consent was obtained before study participation from patients.

Specimens were sectioned (10 μm thickness) and five serial slices were selected. The middle section was stained with hematoxylin and eosin (HE) for histologic comparison and imaged under a standard bright-field light microscope (Eclipse Ci-L; Nikon Corp., Tokyo, Japan) with a CCD (DS-Fi2; Nikon). The remaining four sections were used for MPM imaging.

MPM

MPM was performed using an LSM 510 META imaging system (Carl Zeiss AG, Jena, Germany) equipped with a femtosecond Ti:sapphire laser (110 fs, 76 MHz, Mira 900-F; Coherent Inc., Santa Clara, CA, United States) mode-locked at a wavelength of 810 nm as described previously[17,18]. A Plan-Apochromat oil immersion objective (× 63, numerical aperture = 1.4) was used for image acquisition to collect the backscattered intrinsic SHG and TPEF signals. SHG signals were collected in one channel with a wavelength range of 387-419 nm, and TPEF signals were collected in another channel with a wavelength range of 430-698 nm.

Histopathologic evaluation

TRG from MPM was performed by two independent

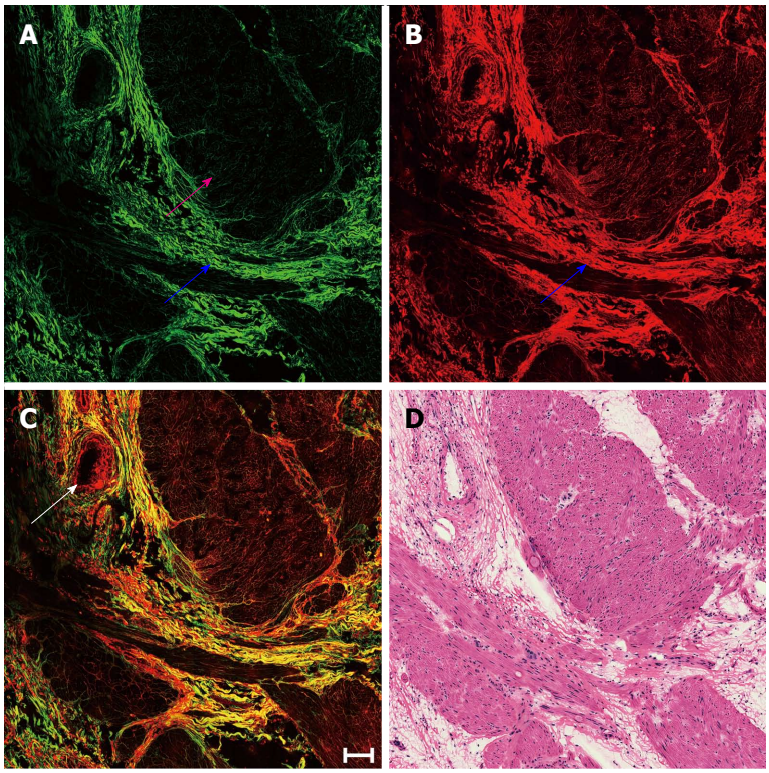

Figure 1 Representative multiphoton microscopy images from a muscularis propria specimen, color-coded green represents the second-harmonic generation signal and color-coded red corresponds to the two-photon excited fluorescence signal. After neoadjuvant radiochemotherapy, predominant fibrosis changes can be seen with minimal inflammatory cells replacing tumor cells. A: Multiphoton microscopy of second-harmonic generation from collagen; B: Two-photon excited fluorescence of collagen, elastin, inflammatory cells, and blood vessels; C: Overlay image (scale bar: 100 μm); D: Hematoxylin and eosin staining of an adjacent section (magnification × 40). The tumor underwent complete regression and was replaced by fibrous tissues (blue arrows). Blood vessel hyperplasia is denoted with a white arrow. Muscular tissue is denoted with a pink arrow.

Table 1 Tumor regression grading scores as determined by histology and microscopy

Patient	Sex	Age (yr)	Cancer classification	TRG score	
				HE	MPM
1	Male	44	Adenocarcinoma	TRG-1	TRG-1
2	Male	38	Adenocarcinoma	TRG-2	TRG-2
3	Female	67	Adenocarcinoma	TRG-2	TRG-2
4	Female	59	Adenocarcinoma	TRG-1	TRG-1
5	Male	59	Adenocarcinoma	TRG-2	TRG-2
6	Male	57	Adenocarcinoma	TRG-3	TRG-3
7	Male	47	Adenocarcinoma	TRG-2	TRG-2

HE: Hematoxylin and eosin staining; TRG: Tumor regression grading; MPM: Multiphoton microscopy.

investigators who were blinded to the results. Tumor regression was classified into five histologic grades according to vital tumor tissue at the ratio of fibrosis[8]: TRG-1: fibrosis without detectable tumor tissue (complete regression); TRG-2: fibrosis with scattered tumor cells; TRG-3: fibrosis and tumor cells with preponderance of fibrosis; TRG-4: fibrosis and tumor cells with preponderance of tumor cells; TRG-5: tumor tissue without regression. TRG scores were confirmed by comparison with corresponding HE-stained sections that were reviewed by a consulting pathologist (Table 1).

Quantification of morphologic features

All quantitative analyses were performed by two individuals experienced in identification of TPEF/SHG images. Collagen and elastin changes and metabolic status after neoadjuvant radiochemotherapy were quantified as SHG/TPEF and redox ratios.

Statistical analysis

One-way analyses of variance were conducted to compare differences using SPSS, version 15.0 statistical software (SPSS Inc., Chicago, IL, United States). Data are presented as mean ± SD, and a P-value < 0.05 was considered significant.

RESULTS

Stromal fibrosis

Representative MPM images depicting the predominant fibrosis with minimal inflammatory cell infiltration replacing large parts of a previous tumor in the muscularis propria following neoadjuvant radiochemotherapy are shown in Figure 1. The smooth muscle was divided due to prior cancer invasion, and the tumor underwent complete regression and was replaced by fibrous tissue mainly composed of collagen fibers, which simultaneously generated SHG (blue

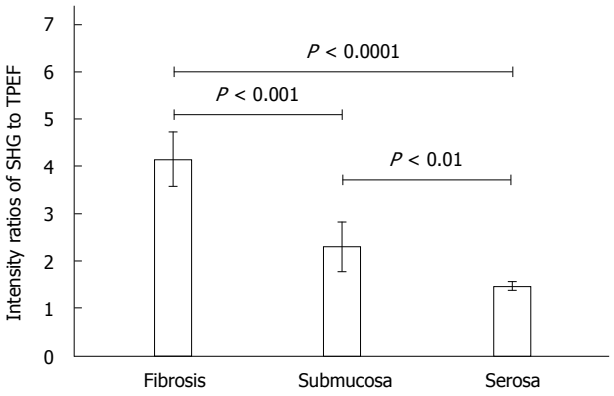

Figure 2 Ratios of second-harmonic generation and two-photon excited fluorescence intensity. Error bars indicate the standard deviation. SHG: Second-harmonic generation; TPEF: Two-photon excited fluorescence.

arrow in Figure 1A) and TPEF (blue arrow in Figure 1B) signals. Additionally, blood vessel hyperplasia was observed as thickening and fibrosis of the intima and media accompanied by minimal inflammatory cell infiltration (white arrow in Figure 1C).

Although fibrosis can easily be differentiated from muscular tissues (pink arrow in Figure 1A) by strong SHG signals, the submucosa and serosa also contain connective tissue comprised of collagen. Therefore, the ratio of SHG to TPEF intensity was calculated to distinguish fibrosis from normal submucosal and serosal tissues, as well as to quantitatively describe the change in fibrous tissue. SHG/TPEF ratio in stromal fibrosis was significantly higher than those in the submucosa and serosa ($P < 0.001$ for both) (Figure 2).

Residual tumors

Representative MPM images depicting remaining malignant glands dispersed within the muscularis propria with minimal inflammatory cell infiltration are shown in Figure 3. Tumor cells in post-treatment rectal carcinoma may show marked changes and these altered tumor cells may become more solid (blue arrows in Figure 3C) or still have a glandular growth pattern (white arrow in Figure 3C). The elastic fibers became fragmented (pink arrows in Figure 3B), while there was an increase in collagen fibers because of stromal fibrosis (Figure 3A).

To further characterize the residual tumors, an image-guide spectral analysis method was used to obtain emission spectra of the cancerous cells, revealing two peaks at 470 and 530 nm (red arrows in Figure 4). These peaks were used to calculate the redox ratio of nicotinamide adenine dinucleotide hydrogen (NADH) to flavin adenine dinucleotide (FAD), represented by fluorescence at 470 and 530 nm, respectively[19,20]. The NADH/FAD ratio was 1.19 ± 0.02.

DISCUSSION

A recent meta-analysis found that partial tumor regression is associated with improvement in disease-free survival[21]. Furthermore, TRG can serve as an independent predictor of disease-free and metastases-free survivals[6,11,22]. The prognostic value of this measure may even exceed that of currently used staging systems (*e.g.*, tumor-node-metastasis staging), which are based on characteristics of untreated tumors[12]. The evaluation criteria of the TRG system incorporate the ratio of tumor cells to fibrosis. However, evaluating TRG using current approaches such as computerized tomography, magnetic resonance imaging, and positron emission computed tomography is challenging, as these medical imaging technologies lack sufficient resolution[23].

MPM relies on nonlinear optical processes to achieve high resolution imaging of biologic tissues, and can detect cellular and subcellular tissue microstructures. Compared with its single-photon counterpart, TPEF offers an inherent optical sectioning property and deep penetration, and the nonlinear scattering from non-centrosymmetric structures provides complementary information to visualize endogenous structures in intact tissues. Residual tumor cells are detected by the TPEF signal, and the SHG signal is used to detect fibrotic tissue. The SHG/TPEF ratio can be used to distinguish fibrosis from submucosal and serosal tissues, as well as to quantify the fibrotic change, which has been proposed as a diagnostic indicator for gastrointestinal diseases[24,25].

Neoadjuvant radiochemotherapy can result in significant morphologic changes, including disrupted muscles, tumor regression, and extensive fibrosis[26]. Moreover, tumor cells in post-treatment rectal carcinoma can become more solid or retain a glandular growth pattern similar to untreated colorectal adenocarcinoma[27]. These alterations were readily detected in the specimens evaluated by MPM in this study. Also observed was the division and damage of muscular tissue by invasion of the adenocarcinoma, which can cause destruction or elimination of collagen and elastic fibers[24].

The redox ratio is an indicator of cellular metabolic state[28]. As this is known to be accelerated in cancerous cells, the redox ratio can be used to quantitatively monitor tumor regression[29]. In this work, the redox ratio in specimens was close to the metabolic state of normal cells[20]. This result supports the notion that abnormal cells undergo significant regression after neoadjuvant radiochemotherapy, which can be assessed *via* MPM.

In conclusion, MPM was used to evaluate TRG in colorectal cancer after neoadjuvant treatment. Spectral analyses provided quantitative evaluations of tumor regression and fibrosis, which corresponded to TRG *via* histopathologic investigation. Given the advantages of this method, including the capacity to produce real-time, label-free images that can be acquired in the near-infrared range, MPM may represent a valuable

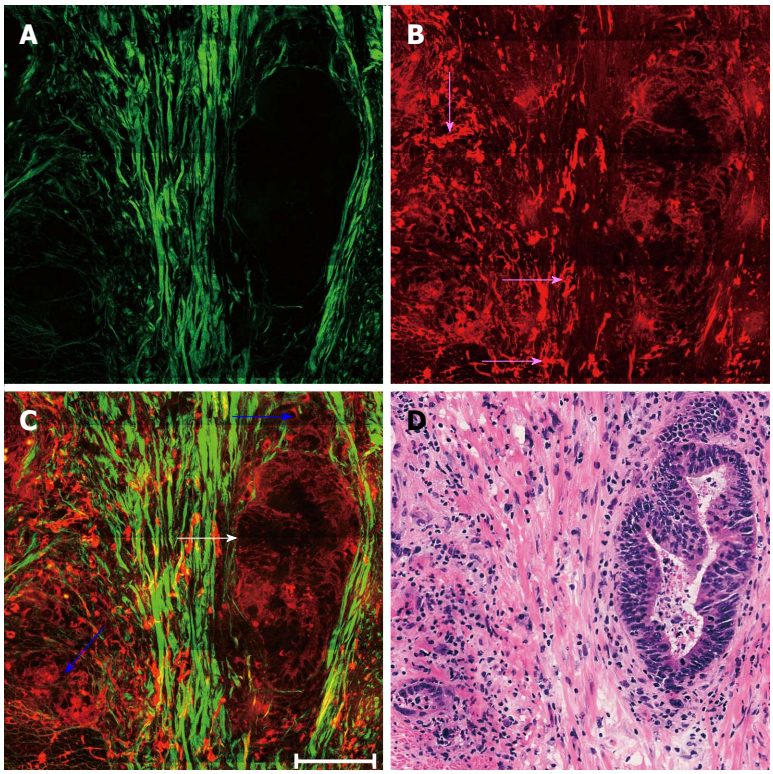

Figure 3 Representative multiphoton microscopy images from a muscularis propria specimen after neoadjuvant radiochemotherapy, color-coded green represents the second-harmonic generation signal and color-coded red corresponds to the two-photon excited fluorescence signal. Remaining malignant glands are dispersed deep within the rectal muscularis propria. A: Multiphoton microscopy of second-harmonic generation from collagen; B: Two-photon excited fluorescence of carcinomatous cells, inflammatory cells, and elastin; C: Overlay image (scale bar: 100 μm); D: Hematoxylin and eosin staining of an adjacent section (magnification × 40). Tumor cells in post-treatment rectal carcinoma show marked changes, and can become more solid (blue arrows) or retain a glandular growth pattern (white arrow). Fragmented elastic fibers are denoted by pink arrows.

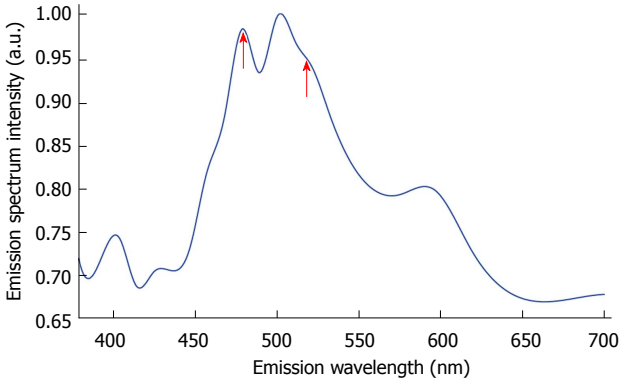

Figure 4 Normalized emission spectrum of residual tumor cells. Excitation wavelength (810 nm) revealed two emission peaks (red arrows).

tool to evaluate TRG after neoadjuvant therapy to treat colorectal carcinoma. This is the first demonstration of the use of MPM to estimate TRG of colorectal carcinoma following neoadjuvant treatment. The results suggest that MPM has a promising future for real-time optical biopsy diagnosis of tumor regression.

COMMENTS

Background

Tumor regression grading (TRG) can provide important prognostic information and should be included in histopathologic reports of colorectal carcinoma after neoadjuvant treatment. However, the resolution of current imaging approaches, such as computerized tomography, magnetic resonance imaging, and positron emission tomography, is insufficient for accurate and easy assessment. The purpose of this study was to evaluate the feasibility of using multiphoton microscopy (MPM) to obtain optical diagnoses for the TRG system.

Research frontiers

The TRG system provides highly valuable prognostic information for evaluating colorectal cancer after neoadjuvant treatment.

Innovations and breakthroughs

This is the first study evaluating the use of MPM for optical diagnosis of tumor regression.

Applications

These results are essential and significant for developing MPM for TRG, and to perform real-time diagnosis of tumor response after neoadjuvant treatment.

Terminology

MPM incorporates nonlinear optical processes to generate second-harmonic generation and two photon excited fluorescence signals.

Peer-review

The article has good characteristics, value, and significance.

REFERENCES

1 **Hwang MR**, Park JW, Park S, Yoon H, Kim DY, Chang HJ, Kim SY, Park SC, Choi HS, Oh JH, Jeong SY. Prognostic impact of circumferential resection margin in rectal cancer treated with preoperative chemoradiotherapy. *Ann Surg Oncol* 2014; **21**: 1345-1351 [PMID: 24468928 DOI: 10.1245/s10434-014-3484-1]

2 **van Hagen P**, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, Richel DJ, Nieuwenhuijzen GA, Hospers GA, Bonenkamp JJ, Cuesta MA, Blaisse RJ, Busch

OR, ten Kate FJ, Creemers GJ, Punt CJ, Plukker JT, Verheul HM, Spillenaar Bilgen EJ, van Dekken H, van der Sangen MJ, Rozema T, Biermann K, Beukema JC, Piet AH, van Rij CM, Reinders JG, Tilanus HW, van der Gaast A. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012; **366**: 2074-2084 [PMID: 22646630 DOI: 10.1056/NEJMoa1112088]

3 **Martin ST**, Heneghan HM, Winter DC. Systematic review and meta-analysis of outcomes following pathological complete response to neoadjuvant chemoradiotherapy for rectal cancer. *Br J Surg* 2012; **99**: 918-928 [PMID: 22362002 DOI: 10.1002/bjs.8702]

4 **Ychou M**, Boige V, Pignon JP, Conroy T, Bouché O, Lebreton G, Ducourtieux M, Bedenne L, Fabre JM, Saint-Aubert B, Genève J, Lasser P, Rougier P. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. *J Clin Oncol* 2011; **29**: 1715-1721 [PMID: 21444866 DOI: 10.1200/JCO.2010.33.0597]

5 **Schrag D**, Weiser MR, Goodman KA, Gonen M, Hollywood E, Cercek A, Reidy-Lagunes DL, Gollub MJ, Shia J, Guillem JG, Temple LK, Paty PB, Saltz LB. Neoadjuvant chemotherapy without routine use of radiation therapy for patients with locally advanced rectal cancer: a pilot trial. *J Clin Oncol* 2014; **32**: 513-518 [PMID: 24419115 DOI: 10.1200/JCO.2013.51.7904]

6 **Abdul-Jalil KI**, Sheehan KM, Kehoe J, Cummins R, O'Grady A, McNamara DA, Deasy J, Breathnach O, Grogan L, O'Neill BD, Faul C, Parker I, Kay EW, Hennessy BT, Gillen P. The prognostic value of tumour regression grade following neoadjuvant chemoradiation therapy for rectal cancer. *Colorectal Dis* 2014; **16**: O16-O25 [PMID: 24119076 DOI: 10.1111/codi.12439]

7 **Rödel C**, Martus P, Papadoupolos T, Füzesi L, Klimpfinger M, Fietkau R, Liersch T, Hohenberger W, Raab R, Sauer R, Wittekind C. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. *J Clin Oncol* 2005; **23**: 8688-8696 [PMID: 16246976 DOI: 10.1200/JCO.2005.02.1329]

8 **Mandard AM**, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, Roussel A, Jacob JH, Segol P, Samama G. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer* 1994; **73**: 2680-2686 [PMID: 8194005 DOI: 10.1002/1097-0142(19940601)73]

9 **Dworak O**, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis* 1997; **12**: 19-23 [PMID: 9112145 DOI: 10.1007/s003840050072]

10 **Becker K**, Mueller JD, Schulmacher C, Ott K, Fink U, Busch R, Böttcher K, Siewert JR, Höfler H. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. *Cancer* 2003; **98**: 1521-1530 [PMID: 14508841 DOI: 10.1002/cncr.11660]

11 **Bouzourene H**, Bosman FT, Seelentag W, Matter M, Coucke P. Importance of tumor regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with preoperative radiotherapy. *Cancer* 2002; **94**: 1121-1130 [PMID: 11920483 DOI: 10.1002/cncr.10327]

12 **Thies S**, Langer R. Tumor regression grading of gastrointestinal carcinomas after neoadjuvant treatment. *Front Oncol* 2013; **3**: 262 [PMID: 24109590 DOI: 10.3389/fonc.2013.00262]

13 **Ying M**, Zhuo S, Chen G, Zhuo C, Lu J, Zhu W, Xie S, Chen J, Yan J. Real-time noninvasive optical diagnosis for colorectal cancer using multiphoton microscopy. *Scanning* 2012; **34**: 181-185 [PMID: 21898461 DOI: 10.1002/sca.20286]

14 **Wu X**, Chen G, Lu J, Zhu W, Qiu J, Chen J, Xie S, Zhuo S, Yan J. Label-free detection of breast masses using multiphoton microscopy. *PLoS One* 2013; **8**: e65933 [PMID: 23755295 DOI: 10.1371/journal.pone.0065933]

15 **Hoover EE**, Squier JA. Advances in multiphoton microscopy technology. *Nat Photonics* 2013; **7**: 93-101 [PMID: 24307915 DOI: 10.1038/nphoton.2012.361]

16 **Campagnola PJ**, Loew LM. Second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms. *Nat Biotechnol* 2003; **21**: 1356-1360 [PMID: 14595363 DOI: 10.1038/nbt894]

17 **Chen J**, Zhuo S, Chen G, Yan J, Yang H, Liu N, Zheng L, Jiang X, Xie S. Establishing diagnostic features for identifying the mucosa and submucosa of normal and cancerous gastric tissues by multiphoton microscopy. *Gastrointest Endosc* 2011; **73**: 802-807 [PMID: 21457819 DOI: 10.1016/j.gie.2010.12.016]

18 **Chen J**, Xu J, Kang D, Xu M, Zhuo S, Zhu X, Jiang X. Multiphoton microscopic imaging of histological sections without hematoxylin and eosin staining differentiates carcinoma in situ lesion from normal oesophagus. *Appl Phys Lett* 2013; **103**: 183701 [DOI: 10.1063/1.4826322]

19 **Monici M**. Cell and tissue autofluorescence research and diagnostic applications. *Biotechnol Annu Rev* 2005; **11**: 227-256 [PMID: 16216779 DOI: 10.1016/S1387-2656(05)11007-2]

20 **Zhuo S**, Chen J, Luo T, Jiang X, Xie S, Chen R. Two-layered multiphoton microscopic imaging of cervical tissue. *Lasers Med Sci* 2009; **24**: 359-363 [PMID: 18536958 DOI: 10.1007/s10103-008-0570-2]

21 **Lee YC**, Hsieh CC, Chuang JP. Prognostic significance of partial tumor regression after preoperative chemoradiotherapy for rectal cancer: a meta-analysis. *Dis Colon Rectum* 2013; **56**: 1093-1101 [PMID: 23929020 DOI: 10.1097/DCR.0b013e318298e36b]

22 **Rubbia-Brandt L**, Giostra E, Brezault C, Roth AD, Andres A, Audard V, Sartoretti P, Dousset B, Majno PE, Soubrane O, Chaussade S, Mentha G, Terris B. Importance of histological tumor response assessment in predicting the outcome in patients with colorectal liver metastases treated with neo-adjuvant chemotherapy followed by liver surgery. *Ann Oncol* 2007; **18**: 299-304 [PMID: 17060484 DOI: 10.1093/annonc/mdl386]

23 **Weissleder R**, Pittet MJ. Imaging in the era of molecular oncology. *Nature* 2008; **452**: 580-589 [PMID: 18385732 DOI: 10.1038/nature06917]

24 **Liu N**, Chen J, Chen G, Yan J, Zhuo S, Jiang X. Detecting the imaging characteristics of colorectal carcinoma invading the muscularis propria with multiphoton microscopy. *Laser Phys Lett* 2012; **9**: 155-159 [DOI: 10.1002/lapl.201110106]

25 **Vidal Bde C**, Mello ML. Optical anisotropy of collagen fibers of rat calcaneal tendons: An approach to spatially resolved supramolecular organization. *Acta Histochem* 2010; **112**: 53-61 [PMID: 18835013 DOI: 10.1016/j.acthis.2008.07.005]

26 **O'Neil M**, Damjanov I. Histopathology of colorectal cancer after neoadjuvant chemoradiation therapy. *Open Pathol J* 2009; **3**: 91-98 [DOI: 10.2174/1874375700903020091]

27 **Shia J**, Guillem JG, Moore HG, Tickoo SK, Qin J, Ruo L, Suriawinata A, Paty PB, Minsky BD, Weiser MR, Temple LK, Wong WD, Klimstra DS. Patterns of morphologic alteration in residual rectal carcinoma following preoperative chemoradiation and their association with long-term outcome. *Am J Surg Pathol* 2004; **28**: 215-223 [PMID: 15043311 DOI: 10.1097/00000478-200402000-00009]

28 **Skala MC**, Riching KM, Gendron-Fitzpatrick A, Eickhoff J, Eliceiri KW, White JG, Ramanujam N. In vivo multiphoton microscopy of NADH and FAD redox states, fluorescence lifetimes, and cellular morphology in precancerous epithelia. *Proc Natl Acad Sci USA* 2007; **104**: 19494-19499 [PMID: 18042710 DOI: 10.1073/pnas.0708425104]

29 **Tiede LM**, Rocha-Sanchez SM, Hallworth R, Nichols MG, Beisel K. Determination of hair cell metabolic state in isolated cochlear preparations by two-photon microscopy. *J Biomed Opt* 2007; **12**: 021004 [PMID: 17477711 DOI: 10.1117/1.2714777]

P- Reviewer: Gassler N **S- Editor**: Qi Y **L- Editor**: Wang TQ
E- Editor: Ma S

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327