Biochemical and Biophysical Research Communications 405 (2011) 37–41

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Detection of ER stress *in vivo* by Raman spectroscopy

Akira Hosoda [a,1], Atsushi Maruyama [b,c,1], Daisuke Oikawa [a,d], Yusuke Oshima [b,e], Yuichi Komachi [f], Genichi Kanai [f], Hidetoshi Sato [b,g], Takao Iwawaki [a,h,*]

[a] *Iwawaki Initiative Research Unit, Advanced Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*
[b] *Optical Biopsy Development Research Unit, Advanced Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*
[c] *Top Runner Incubation Center for Academia-Industry Fusion, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan*
[d] *Research Fellow of the Japan Society for the Promotion of Science, 8 Ichiban-cho, Chiyoda-ku, Tokyo 102-8472, Japan*
[e] *Department of Imaging Science, National Institute for Basic Biology, 38, Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan*
[f] *Machida Endoscope Co. Ltd., 6-13-8 Honkomagome Bunkyo-ku, Tokyo 113-0021, Japan*
[g] *Department of Bioscience, Kwansei-Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337, Japan*
[h] *PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan*

ARTICLE INFO

Article history:
Received 16 December 2010
Available online 30 December 2010

Keywords:
ER stress
Raman spectroscopy

ABSTRACT

The endoplasmic reticulum (ER) is an organelle in which most membrane and secretory proteins are synthesized. If these proteins are not folded correctly, unfolded proteins accumulate in the ER lumen, causing a cellular situation known as ER stress. Recently, many studies on the relationship between ER stress and diseases have been reported. Thus, studies of ER stress *in vivo* should yield information that is useful in pathology. Model mice have been developed as a powerful tool to visualize ER stress *in vivo*, but this approach depends on transgenic technology. Here, we report on a method of detecting ER stress *in vivo* by Raman spectroscopy. Our experiments revealed that two specific Raman bands were reduced in both cultured cells and animal tissues in an ER stress dependent manner. This suggests that Raman spectroscopy could be a useful tool to detect ER stress *in vivo* without transgenic technology.

1. Introduction

The endoplasmic reticulum (ER) is an organelle whose main function is to synthesize secretory and membrane proteins. These proteins are initially located in the ER as unfolded and unmodified nascent polypeptides and are then correctly folded, assembled and modified by numerous molecular chaperones and catalysts that are resident in the ER lumen [1,2]. However, in some cases, the proteins accumulate as unfolded and malfolded proteins in the ER lumen. This cellular situation is known to cause ER stress, which perturbs ER functions [3].

There have been many studies on the relationship between ER stress and diseases [4]. For example, some cancer studies have shown that ER stress is caused during tumor growth [5,6] and that cellular responses to ER stress contribute to tumor growth [7,8]. Some diabetes studies have shown that ER stress is involved in apoptotic cell death of pancreatic β cells [9] and that cellular responses to ER stress contribute to insulin biosynthesis [10,11]. Meanwhile, in some studies of neurodegenerative diseases, ER stress was closely associated with the neuronal cell death caused by Alzheimer's disease [12], Parkinson's disease [13], and polyglutamine diseases [14]. Therefore, studies of ER stress *in vivo* should yield information that is useful in pathology.

In order to detect ER stress *in vivo*, Iwawaki et al. previously developed model mice [15,16]. These mice can emit fluorescent or luminescent signals depending on the activity of molecules that respond to ER stress, and thus facilitate the detection of ER stress: ER stress can be detected as fluorescent or luminescent signals without lysis of cells and tissues. In fact, they reported the ER stress condition of the pancreas, skeletal muscle, salivary glands, and placenta by *in vivo* imaging with living ERAI (ER stress activated indicator) mice [15–17]. However, a disadvantage of the ERAI system is that it depends on transgenic technology; ER stress cannot be detected without introducing artificially-designed reporter genes into animals or cells. Therefore, the ERAI system cannot be used for detecting ER stress in the human body.

Raman measurements with biological samples have been studied intensively in recent years, such as a cultured human lung cancer single cell [18], human stomach cancer tissues obtained during biopsy [19], *in vivo* rat upper digestive tract under endoscopic surveillance [20], and *in vivo* rat cerebral neocortex undergoing

Abbreviations: ER, endoplasmic reticulum; ERAI, ER stress activated indicator.

* Corresponding author at: Iwawaki Initiative Research Unit, Advanced Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. Fax: +81 48 467 8503.
E-mail address: iwawaki@riken.jp (T. Iwawaki).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.12.112

craniotomy [21]. Since these studies by Raman spectroscopy are based on measurements of a specific scattering of monochromatic light that corresponds to the state of an endogenous biological molecule, Raman measurements can be used to analyze living cells and animals without requiring a staining process or introducing any exogenous reporter gene. Here, we examine the possibility of detecting ER stress in living cells and animals by Raman spectroscopy as a pilot study in order to establish methods without gene modification in the near future.

2. Materials and methods

2.1. Cell culture, treatment and preparations

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (4.5 g/l glucose) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum at 37 °C in 5% CO_2. For Raman measurement, cells were trypsinized and washed once with PBS to remove Phenol-Red, and suspended in DMEM not containing Phenol-Red and seeded on 35-mm fused-silica glass bottom dishes. After 24 h, cells were treated with 5 μg/ml tunicamycin, 800 nM thapsigargin, or 100 μg/ml etoposide, or cultured at 42 °C for the indicated time periods. After treatment, cells were rinsed twice with Hanks' balanced salt solution not containing Phenol-Red, and then subjected to Raman measurement.

2.2. Animals

Eight-week-old female C57BL/6 mice were used in this study. We intraperitoneally injected these mice with tunicamycin (500 ng/g body weight) or the same volume of saline as a control. At 18 h after drug treatment, we fixed the distal extremities of the mice under anesthesia with Nembutal (Dainippon Sumitomo Pharma), conducted an abdominal median incision, and then performed Raman measurement of tissues in a dark room. During measurement, anesthetic depth was maintained. Three mice were used for each treatment. Experimental protocols involving animals were approved by the Animal Studies Committees at RIKEN.

2.3. Raman measurement of cultured cells and animal tissues

Raman spectra for cultured cells were obtained using the Nanofinder (Tokyo Instruments) equipped with a 600 lines/mm holographic grating blazed at 750 nm. An excitation laser of 632.8 nm with power of 50 mW was focused on a single cell via a 60 X Water-immersion objective lens with numerical aperture (NA) of 1.2. Raman scattering light from the sample was collected using the same objective lens for the visible region and a 60 s exposure time. The spectral resolution was less than 2 cm^{-1}, and the diameter of the laser spot at the focal point was less than 1 μm. The number of Raman spectra randomly taken in each cell-culture dish was described in figure legends. Background spectra were obtained by focusing on a vacant region on each dish where no cell existed. A reference spectrum was obtained without laser irradiation by placing a white light source at the focal point.

Raman spectra for animal tissues were obtained using the experimental system of a micro Raman probe (Machida Endoscope) [22,23]. The micro Raman probe had a nose-mounted sapphire ball lens of 0.5 mm in diameter (Edmund Optics; NT46-117) [24] and narrow optical fiber of 0.6 mm in diameter (Machida Endoscope). The excitation light from a 785-nm diode laser (EnWave Optronics; FSL-785-400MM) with a BP filter (Semrock, Rochester; LL01-785-12.5) was focused onto the micro Raman probe excitation fiber by a lens (f = 20 mm). The laser was used with power of 80 mW. Raman scattering light from animal tissues were collected for 300 s. At the detection end, the collection fibers were arrayed linearly to match the input slit of a spectrometer (Kaiser Optical Systems; HoloSpec F/1.8). The light emerging from the fibers was collimated with a lens (f = 15 mm, diameter = 9 mm). The light passing through an LP filter (Semrock, Rochester; LP01-785RS-25) was then focused onto the slit of the spectrometer. The light dispersed by the transmission grating was detected with a CCD detector (Andor Technology; DU401A-BR-DD) with a resolution of 8 cm^{-1} using a 0.1-mm slit. Background spectra were obtained with an aluminum foil-wrapped micro Raman probe, but the end of the probe was not in contact with the foil. A reference spectrum was obtained without laser irradiation by placing a white light source at the focal point.

2.4. Data analysis and statistics

The pure spectrum of each sample, $Y_{pure}(\nu)$, was calculated as $\{Y_{raw}(\nu)-Y_{back}(\nu)\}/Y_{ref}(\nu)$, where $Y_{raw}(\nu)$ is a raw Raman spectrum, $Y_{back}(\nu)$ is a background spectrum and $Y_{ref}(\nu)$ is a reference spectrum. The baseline-corrected Raman spectrum of each sample was then calculated as $\{Y_{pure}(\nu)-Y_{base}(\nu)\}$, in which $Y_{base}(\nu)$ is a fitted polynomial curve constructed as follows. (i) For a spectrum truncated between the minimum Raman shift position ν_{min} and the maximum position ν_{max}, the degree of the function was selected to fit the baseline using a polynomial function. (ii) Using the least squares method, the polynomial function $Y_{base}(\nu)$ was first fitted to the pure Raman spectrum $Y_{pure}(\nu)$. (iii) The pure Raman spectrum $Y_{pure}(\nu)$ was divided into upper and lower parts, relative to the fitted baseline $Y_{base}(\nu)$. (iv) The number of data points on the upper side of $Y_{pure}(\nu)$ was designated NA, and the number in the lower part of $Y_{pure}(\nu)$ was designated NB. If NA < NB, the upper part of $Y_{pure}(\nu)$ was removed from the whole of $Y_{pure}(\nu)$, and the pure Raman spectrum $Y_{pure}(\nu)$ was replaced with the lower part of the pure spectrum. Then, procedure (ii) was repeated. When NA $\geqslant$ NB, the baseline was considered the best fit and optimal. After correction for baseline, the intensity of Raman spectrum of each sample was normalized to that of Raman band of 1004 cm^{-1}. Typically, a sharply-peaked Raman band near 1004 cm^{-1} indicated phenylalanine and was used as the internal standard. The normalized spectrum was used for statistical analysis.

3. Results

First, we measured Raman spectra of cultured cells under the ER stress condition or non-ER stress condition to examine whether ER stress has specific effects on Raman spectra detected from cultured cells. These measurements of cultured cells were performed in the ER-rich region near, but not including, the nucleus, to collect signals from ER as much as possible (Supplementary Fig. S1). In this experiment, we used tunicamycin (inhibitor of N-linked glycosylation) and thapsigargin (releaser of Ca^{2+} from the ER) as an ER stressor, and etoposide (inducer of DNA damage) and culturing cells at 42 °C (heat shock stress) as a non-ER stressor. The effect of tunicamycin and thapsigargin was confirmed by gene induction of BiP, while that of heat shock and etoposide was confirmed by gene induction of HSP70 and chromatin condensation, respectively (Supplementary Fig. S2). Fig. 1A shows the average of Raman spectra of cells under the ER stress condition or non-ER stress condition. We could detect the peaks derived from protein associated with the Raman fingerprint region (Phe: 1004 cm^{-1}, amide III: 1250–1340 cm^{-1}, CH_2: 1440–1460 cm^{-1}, amide I: 1645–1655 cm^{-1}), but not nucleic acid (G: 1320 cm^{-1}, A, G: 1342 cm^{-1}, A, G, T: 1373 cm^{-1}, A, G: 1578 cm^{-1}) [25–28], in a similar manner from all the

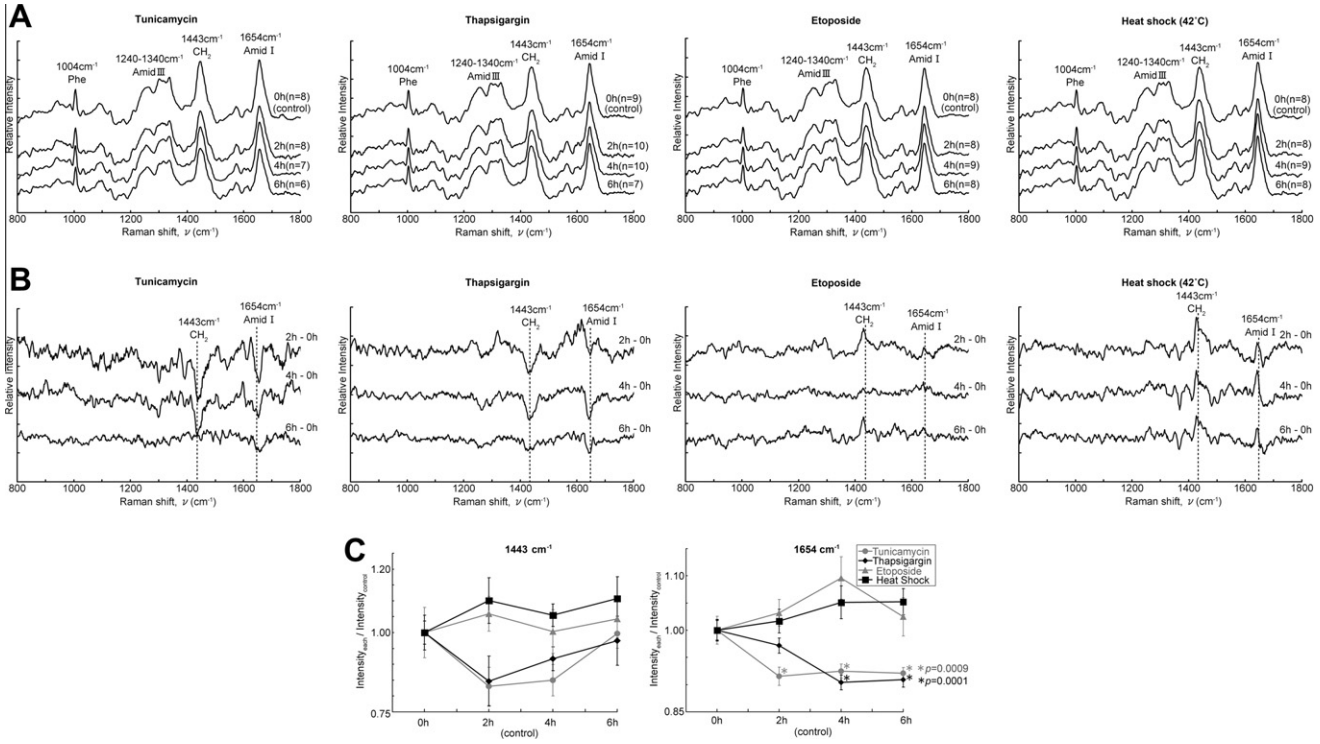

Fig. 1. An ER stress specific decrease of Raman signals in cultured cells. (A) Average Raman spectra under stress conditions. Each spectrum is derived from cells treated with tunicamycin, thapsigargin, heat (42 °C) shock, or etoposide for the indicated period. Each spectrum was normalized to its band of 1004 cm^{-1}. The number of the spectra of control, 2 h, 4 h and 6 h in each treatment are indicated. (B) Subtracted average Raman spectra of cultured cells under stress conditions. Each spectrum of control cells was subtracted from each spectrum of cells exposed to stress indicated for 2 h, 4 h, or 6 h. Each spectrum of control cells and stress-treated cells used in this analysis is derived from B. (C) Ratio of Raman bands of 1443 cm^{-1} and 1654 cm^{-1}. The plotted data are derived from B. The plots and error bars denote mean ± s.e.m. Statistical significance of differences between control and ER stress was determined by Tukey's method.

data in Fig. 1A. This result indicates that we could collect data reproducibly. Fig. 1B shows subtracted Raman spectra between the stress and normal conditions to focus on the effects of cellular stress on Raman spectra. From this analysis, a decrease of Raman signals on 1443 cm^{-1} and 1654 cm^{-1} was observed in the cells treated with ER stressor (tunicamycin and thapsigargin), but not in those treated with non-ER stressor (etoposide and 42 °C). Interestingly, Raman signals on 1443 cm^{-1} of cells treated with ER stressors for 6 h had the same level as those of cells under the normal condition, but we cannot explain the reason. Fig. 2C presents Raman signals on 1443 and 1654 cm^{-1} for ease of understanding.

Next, we measured Raman spectra in tissues and organs of a living animal under the ER stress condition or normal condition to examine whether the decrease of specific Raman signals can be observed even in tissues and organs of an animal similar to cultured cells. Raman spectra were measured in the rectus abdominis

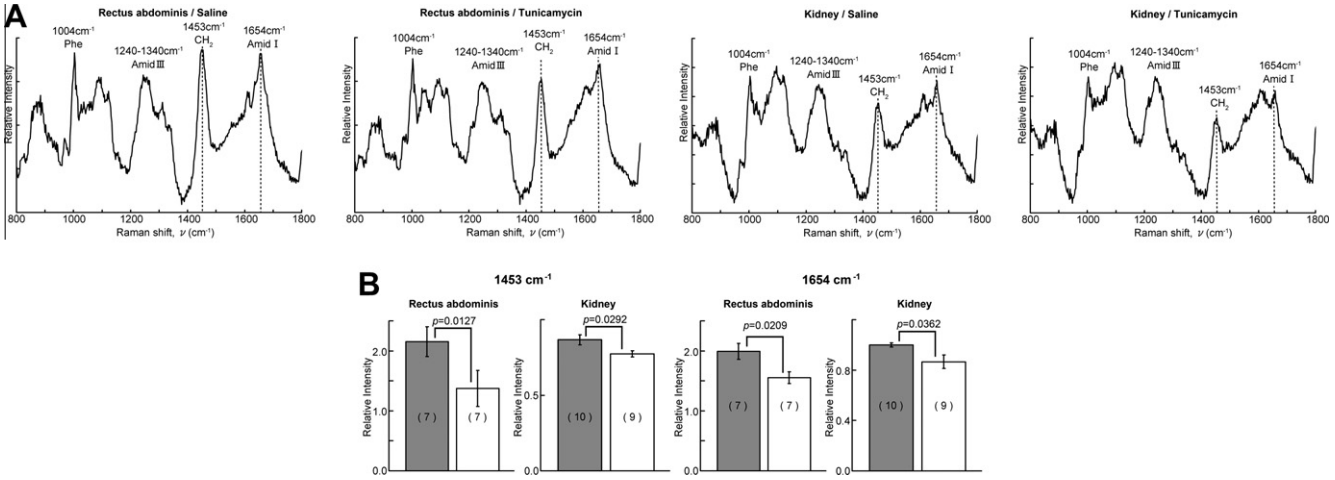

Fig. 2. An ER stress specific decrease of Raman signals in animal tissues. (A) Average Raman spectra of the rectus abdominis and the kidney under the normal (saline) or ER stress condition (tunicamycin). Each spectrum was normalized to its band of 1004 cm^{-1}. The number of the spectra of rectus abdominis/saline, rectus abdominis/tunicamycin, kidney/saline and kidney/tunicamycin are 7, 7, 10 and 9, respectively. (B) Average Raman bands of 1453 and 1654 cm^{-1} of the rectus abdominis and the kidney under the normal (gray column) or ER stress condition (white column). Each histogram and error bar denotes mean ± s.e.m. The number of measurements for each group is indicated in parentheses in each column. Statistical significance of differences was determined by two-tailed Welch's *t*-test or Student's *t*-test.

and the kidney of 8-week-old female mice. Tunicamycin (as an ER stressor) or saline (as a control) was administered by intraperitoneal injection 18 h before measurement of Raman spectra.

Fig. 2A shows the average of Raman spectra of the rectus abdominis and the kidney under the ER stress condition or normal condition. All data were collected reproducibly. Fig. 2B presents Raman signals on 1453 and 1654 cm^{-1} to compare each signal level between under the ER stress condition and normal condition. As expected, the data revealed a decrease of Raman signals on 1453 and 1654 cm^{-1} in both the rectus abdominis and the kidney under the ER stress condition similar to cultured cells.

4. Discussion

In this study, our purpose was to examine the possibility of detecting ER stress in living cells and animals by Raman spectroscopy. Analysis by Raman spectroscopy revealed that Raman signals on 1443/1453 and 1654 cm^{-1} were decreased in an ER stress dependent manner both in living cells and in tissues of living animals (Figs. 1A and 2B). This result indicates the possibility that ER stress-induced cellular effects are detected as changes of specific Raman spectra.

Why were Raman signals on 1443/1453 and 1654 cm^{-1} decreased in cells and tissues under the ER stress condition? We do not have a clear answer to this question yet. It is believed that ER stress is caused by the accumulation of unfolded or malfolded proteins in the ER. It is also known that eukaryotic cells initiate adaptive responses during ER stress [3]. Regarding the molecular mechanism of the response to ER stress in mammalian cells, ATF6, IRE1, and PERK function as sensors for ER stress and are activated via processing or *trans*-autophosphorylation [29]. The phosphorylated IRE1 induces the unconventional splicing of XBP1 mRNA, which is then translated into a functional protein [30,31]. The phosphorylated PERK induces the phosphorylation of eIF2α and the translational activation of ATF4 [32]. Under the ER stress condition, ATF6 is cleaved by Sites-1 and 2 proteases, and its cytoplasmic domain is translocated to the nucleus [33,34]. All of XBP1, ATF4, and cleaved ATF6 work as transcription factors to increase molecular chaperones and catalysts that are resident in the ER [35]. Considering these findings, it is possible that the decrease of Raman signals on 1443/1453 and 1654 cm^{-1} might reflect any conformational change of ER proteins and/or any qualitative or quantitative change of molecules that respond to ER stress. On the other hand, it is also possible that any structural change of the ER itself might be reflected in Raman signals on 1443/1453 and 1654 cm^{-1}, because the ER expands during ER stress [36,37].

A method for detecting ER stress in living animals without transgenic technology would be very useful, because it could be used to detect ER stress in humans. As described in the Introduction, there have been many studies on the relationship between ER stress and diseases, and so monitoring ER stress may be a useful way of diagnosing those diseases. For such diagnosis, low invasiveness is a key factor. In this regard, Raman spectroscopy has several advantages: very fine probes are available for Raman spectroscopy (the probe used in this study was 0.6 mm in diameter) and harmless near-infrared light or visible light, not harmful ultraviolet light, can be used as the irradiating laser light. Raman spectroscopy may also be beneficial for detecting other stresses such as hypoxic stress [38–45] and oxidative stress [46], because these are reported to be associated with various diseases similar to ER stress. On the other hand, this study identified one problem in Raman spectroscopy. When we tried to measure the Raman spectra of not only the rectus abdominis and kidney but also the liver, pancreas, and spleen, the change of Raman signals on 1443/1453 and 1654 cm^{-1} could not be detected in the liver, pancreas, or spleen because of high background signals. To overcome this problem, it is necessary to improve the conditions of measuring Raman spectra in each organ.

Acknowledgments

We thank Ryoko Akai for technical assistance. This work was supported by grants from RIKEN (to T.I.), JST (to T.I.), and MEXT (No. 21790218 to T.I and No. 22113524 to TI).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.12.112.

References

[1] L. Ellgaard, A. Helenius, Quality control in the endoplasmic reticulum, Nat. Rev. Mol. Cell Biol. 4 (2003) 181–191.
[2] T. Anelli, R. Sitia, Protein quality control in the early secretory pathway, EMBO J. 27 (2008) 315–327.
[3] M. Schroder, R.J. Kaufman, ER stress and the unfolded protein response, Mutat. Res. 569 (2005) 29–63.
[4] H. Yoshida, ER stress and diseases, FEBS J. 274 (2007) 630–658.
[5] G. Gazit, J. Lu, A.S. Lee, De-regulation of GRP stress protein expression in human breast cancer cell lines, Breast Cancer Res. Treat. 54 (1999) 135–146.
[6] M.S. Song, Y.K. Park, J.H. Lee, K. Park, Induction of glucose-regulated protein 78 by chronic hypoxia in human gastric tumor cells through a protein kinase C-epsilon/ERK/AP-1 signaling cascade, Cancer Res. 61 (2001) 8322–8330.
[7] B. Drogat, P. Auguste, D.T. Nguyen, M. Bouchecareilh, R. Pineau, J. Nalbantoglu, R.J. Kaufman, E. Chevet, A. Bikfalvi, M. Moenner, IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo, Cancer Res. 67 (2007) 6700–6707.
[8] D. Dong, M. Ni, J. Li, S. Xiong, W. Ye, J.J. Virrey, C. Mao, R. Ye, M. Wang, L. Pen, L. Dubeau, S. Groshen, F.M. Hofman, A.S. Lee, Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development, Cancer Res. 68 (2008) 498–505.
[9] S. Oyadomari, K. Takeda, M. Takiguchi, T. Gotoh, M. Matsumoto, I. Wada, S. Akira, E. Araki, M. Mori, Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway, Proc. Natl. Acad. Sci. USA 98 (2001) 10845–10850.
[10] K.L. Lipson, S.G. Fonseca, S. Ishigaki, L.X. Nguyen, E. Foss, R. Bortell, A.A. Rossini, F. Urano, Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1, Cell Metab. 4 (2006) 245–254.
[11] K.L. Lipson, R. Ghosh, F. Urano, The role of IRE1alpha in the degradation of insulin mRNA in pancreatic beta-cells, PLoS One 3 (2008) e1648.
[12] T. Katayama, K. Imaizumi, N. Sato, K. Miyoshi, T. Kudo, J. Hitomi, T. Morihara, T. Yoneda, F. Gomi, Y. Mori, Y. Nakano, J. Takeda, T. Tsuda, Y. Itoyama, O. Murayama, A. Takashima, P. St. George-Hyslop, M. Takeda, M. Tohyama, Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response, Nat. Cell Biol. 1 (1999) 479–485.
[13] Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno, R. Takahashi, An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin, Cell 105 (2001) 891–902.
[14] H. Nishitoh, A. Matsuzawa, K. Tobiume, K. Saegusa, K. Takeda, K. Inoue, S. Hori, A. Kakizuka, H. Ichijo, ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats, Genes Dev. 16 (2002) 1345–1355.
[15] T. Iwawaki, R. Akai, K. Kohno, M. Miura, A transgenic mouse model for monitoring endoplasmic reticulum stress, Nat. Med. 10 (2004) 98–102.
[16] T. Iwawaki, R. Akai, S. Yamanaka, K. Kohno, Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability, Proc. Natl. Acad. Sci. USA 106 (2009) 16657–16662.
[17] A. Hosoda, M. Tokuda, R. Akai, K. Kohno, T. Iwawaki, Positive contribution of ERdj5/JPDI to endoplasmic reticulum protein quality control in the salivary gland, Biochem. J. 425 (2010) 117–125.
[18] Y. Oshima, H. Shinzawa, T. Takenaka, C. Furihata, H. Sato, Discrimination analysis of human lung cancer cells associated with histological type and malignancy using Raman spectroscopy, J. Biomed. Opt. 15 (2010) 017009.
[19] T. Kawabata, T. Mizuno, S. Okazaki, M. Hiramatsu, T. Setoguchi, H. Kikuchi, M. Yamamoto, Y. Hiramatsu, K. Kondo, M. Baba, M. Ohta, K. Kamiya, T. Tanaka, S. Suzuki, H. Konno, Optical diagnosis of gastric cancer using near-infrared multichannel Raman spectroscopy with a 1064°nm excitation wavelength, J. Gastroenterol. 43 (2008) 283–290.
[20] Y. Hattori, Y. Komachi, T. Asakura, T. Shimosegawa, G. Kanai, H. Tashiro, H. Sato, In vivo Raman study of the living rat esophagus and stomach using a micro-Raman probe under an endoscope, Appl. Spectrosc. 61 (2007) 579–584.

[21] H. Sato, Y.S. Yamamoto, A. Maruyama, T. Katagiri, Y. Matsuura, Y. Ozaki, Raman study of brain functions in live mice and rats: a pilot study, Vib. Spectrosc. 50 (2009) 125–130.
[22] Y. Komachi, T. Katagiri, H. Sato, H. Tashiro, Improvement and analysis of a micro Raman probe, Appl. Opt. 48 (2009) 1683–1696.
[23] Raman Scattering Measurement Device (WO/2010/024397).
[24] J.T. Motz, M. Hunter, L.H. Galindo, J.A. Gardecki, J.R. Kramer, R.R. Dasari, M.S. Feld, Optical fiber probe for biomedical Raman spectroscopy, Appl. Opt. 43 (2004) 542–554.
[25] N. Uzunbajakava, A. Lenferink, Y. Kraan, E. Volokhina, G. Vrensen, J. Greve, C. Otto, Nonresonant confocal Raman imaging of DNA and protein distribution in apoptotic cells, Biophys. J. 84 (2003) 3968–3981.
[26] I. Notingher, S. Verrier, S. Haque, J.M. Polak, L.L. Hench, Spectroscopic study of human lung epithelial cells (A549) in culture: living cells versus dead cells, Biopolymers 72 (2003) 230–240.
[27] I. Notingher, I. Bisson, A.E. Bishop, W.L. Randle, J.M. Polak, L.L. Hench, *In situ* spectral monitoring of mRNA translation in embryonic stem cells during differentiation *in vitro*, Anal. Chem. 76 (2004) 3185–3193.
[28] J. Stepanek, M. Vincent, P.Y. Turpin, D. Paulin, S. Fermandjian, B. Alpert, C. Zentz, C → G base mutations in the CArG box of c-fos serum response element alter its bending flexibility. Consequences for core-SRF recognition, FEBS J. 274 (2007) 2333–2348.
[29] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[30] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell 107 (2001) 881–891.
[31] M. Calfon, H. Zeng, F. Urano, J.H. Till, S.R. Hubbard, H.P. Harding, S.G. Clark, D. Ron, IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA, Nature 415 (2002) 92–96.
[32] H.P. Harding, I. Novoa, Y. Zhang, H. Zeng, R. Wek, M. Schapira, D. Ron, Regulated translation initiation controls stress-induced gene expression in mammalian cells, Mol. Cell 6 (2000) 1099–1108.
[33] K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, Mol. Biol. Cell 10 (1999) 3787–3799.
[34] J. Ye, R.B. Rawson, R. Komuro, X. Chen, U.P. Davé, R. Prywes, M.S. Brown, J.L. Goldstein, ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs, Mol. Cell 6 (2000) 1355–1364.
[35] Y. Ma, L.M. Hendershot, The unfolding tale of the unfolded protein response, Cell 107 (2001) 827–830.
[36] R. Sriburi, S. Jackowski, K. Mori, J.W. Brewer, XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum, J. Cell Biol. 167 (2004) 35–41.
[37] H. Bommiasamy, S.H. Back, P. Fagone, K. Lee, S. Meshinchi, E. Vink, R. Sriburi, M. Frank, S. Jackowski, R.J. Kaufman, J.W. Brewer, ATF6alpha induces XBP1-independent expansion of the endoplasmic reticulum, J. Cell Sci. 122 (2009) 1626–1636.
[38] G.L. Semenza, Hypoxia and human disease, J. Mol. Med. 85 (2007) 1293–1294.
[39] C.T. Taylor, S.P. Colgan, Hypoxia and gastrointestinal disease, J. Mol. Med. 85 (2007) 1295–1300.
[40] M.C. Brahimi-Horn, J. Chiche, J. Pouysségur, Hypoxia and cancer, J. Mol. Med. 85 (2007) 1301–1307.
[41] R.V. Shohet, J.A. Garcia, Keeping the engine primed: HIF factors as key regulators of cardiac metabolism and angiogenesis during ischemia, J. Mol. Med. 85 (2007) 1309–1315.
[42] R.M. Tuder, J.H. Yun, A. Bhunia, I. Fijalkowska, Hypoxia and chronic lung disease, J. Mol. Med. 85 (2007) 1317–1324.
[43] M. Nangaku, K.U. Eckardt, Hypoxia and the HIF system in kidney disease, J. Mol. Med. 85 (2007) 1325–1330.
[44] R.R. Ratan, A. Siddiq, N. Smirnova, K. Karpisheva, R. Haskew-Layton, S. McConoughey, B. Langley, A. Estevez, P.T. Huerta, B. Volpe, S. Roy, C.K. Sen, I. Gazaryan, S. Cho, M. Fink, J. LaManna, Harnessing hypoxic adaptation to prevent, treat, and repair stroke, J. Mol. Med. 85 (2007) 1331–1338.
[45] A.S. Zinkernagel, R.S. Johnson, V. Nizet, Hypoxia inducible factor (HIF) function in innate immunity and infection, J. Mol. Med. 85 (2007) 1339–1346.
[46] A. Spector, Review: oxidative stress and disease, J. Ocul. Pharmacol. Ther. 16 (2000) 193–201.