学術論文抄録—2011 年発表

An Image Stabilization Technology for Digital Still Camera Based on Blind Deconvolution

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IEICE TRANS. INF. & SYST., VOL.E94-D, NO.5, pp. 1082-1089, May 2011

In this article, we present a new image-stabilization technology for still images based on blind deconvolution and introduce it to a consumer digital still camera. This technology consists of three features: (1) double-exposure-based PSF detection, (2) efficient image deblurring filter, and (3) edge-based ringing reduction. Without deteriorating the deblurring performance, the new technology allows us to reduce processing time and ringing artifacts, both of which are common problems in image deconvolution.

Automated Extraction of Non <h>-tagged Headers in Webpages by Decision Trees

H. Okada* and H. Arakawa

Proc. of International Conference on Instrumentation, Control and Information Technology (SICE Annual Conference 2011), pp.2117-2120 (2011.09)

The guideline #5.2a in the JIS X 8341-3 reccomends to “represent headings with heading elements instead of difference in font size, etc”. Thus, in checking webpage accessibility, headers that are not tagged with heading tags (<h1>-<h6>) should be extracted as problems. In this paper, we propose a method for the extraction. Our idea is to let a machine learning method to automatically derive decision rules from problem instances on the web. We define 26 attributes of HTML elements for deriving decision trees. Values of these attributes are calculated by parsing the HTML source of the webpage. Accuracy of our method was evaluated by 10-fold cross validations with the data we collected from the web. The accuracy was 85-88% in average in terms of F-measure. Non <h>-tagged image headers were slightly better discriminated than non <h>-tagged text headers.

Application and Evaluation of Augmented Reality User Interface to a Card Game “Scopa”

H. Okada* and T. Matsuse

Proc. of International Conference on Instrumentation, Control and Information Technology (SICE Annual Conference 2011), pp.2127-2130 (2011.09)

We propose an augmented reality application system for playing Scopa. The idea of this application is that visual augmentation by means of the AR method will help a Scopa beginner play games, learn rules from his/her game experiences under the support of the system, and thus become an experienced player who can play without any support. The system captures cards in the player's view via a camera, recognizes the current state, and visually augments cards to guide the user perform appropriate actions (e.g., discard an unwanted card). Our implementation and evaluation of the system are reported in this paper.

Evolving RoboCup Soccer Player Formations by Particle Swarm Optimization

H. Okada*, T. Wada and A. Yamashita

Proc. of International Conference on Instrumentation, Control and Information Technology (SICE Annual Conference 2011), pp.1951-1953 (2011.09)

Researchers have applied evolutionary algorithms to RoboCup Soccer players/teams and evaluate the effectiveness of the algorithm in evolving good players and teams, but further investigations are required to know more about the ability of the algorithms. We report our application of particle swarm optimization (PSO) to the evolution of RoboCup Soccer player formations: how well formations for various team performances (e.g., offensive, defensive, balanced) can be automatically obtained by means of PSO.

人と情報の検索および相互作用を目指したソーシャルサーチシステムの研究開発

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日本ソフトウェア科学会「コンピュータソフトウェア」 Vol.28, No.4 (2011), pp.196-205

本研究では,「情報と人」を瞬時に発見することによって，検索サービスとソーシャルサービスの双方の利点を活用できる新たな検索システムを提案する．本システムの検索機能では，検索結果にリアルタイムでの閲覧者数が表示され，ユーザはページと同時に閲覧者も発見できる．また，コミュニケーション機能により，同一ページを閲覧しているユーザとページを通して，その時そのページ上でコミュニケーションが行える．これにより，ページの情報だけでなく，人が持っている知識をも獲得できる可能性が高まる．さらに，コミュニケーションにより発生したページ内での会話ログも参照でき，他者の類似の質問や回答があった場合，問合せの手間が省ける．本稿では，提案システムの実現のための設計および実装について述べる．

新聞記事を対象とするテキスト印象マイニング手法の設計と評価

熊本　忠彦・河合由起子*・田中　克己

電子情報通信学会論文誌 D Vol. J94-D No. 3 pp. 540-548

著者らは，コンテンツを見たり聞いたりしたときに人々が感じる印象をコンテンツそのものから抽出する手法の研究開発を行っている．本論文では，新聞記事を例として取り上げ，記事を読んだ人々が感じる印象を記事そのものから抽出するテキスト印象マイニング手法を提案する．具体的には，新聞記事データベースを解析し，記事に現れる各単語が記事の印象に及ぼす影響を数値化した印象辞書を構築するとともに，この印象辞書を用いて記事の印象値を算出する手法（算出法）を開発する．さらに，この算出法が記事から算出する印象値と人々がその記事を読んだときに感じる印象値との対応関係を回帰分析により調べ，その結果得られる回帰式を用いて算出した印象値を補正するという方法で高精度なテキスト印象マイニングを実現する．但し，提案手法により抽出される印象は,「楽しい⇔悲しい」,「うれしい⇔怒り」,「のどか⇔緊迫」の 3 種類であり，それぞれの印象に対し 7 段階の評価尺度（印象尺度）を設定している．提案手法の有効性を検証するために行った被験者実験では，それぞれの印象尺度における平均誤差が 0.69，0.49，0.64 となり，特に「うれしい⇔怒り」に対しては高い精度を得ている．

Page History Explorer: Visualizing and Comparing Page Histories

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IEICE Transactions on Information and Systems, Special Issue on Data Engineering, pp. 564-577 (2011)

Due to the increased preservation efforts, large amounts of past Web data have been stored in Web archives and other archival repositories. Utilizing this data can offer certain benefits to users, for example, it can facilitate page understanding. In this paper, we propose a system for interactive exploration of page histories. We demonstrate an application called Page History Explorer (PHE) for summarizing and visualizing histories of Web pages. PHE portrays the overview of page evolution, characterizes its typical content over time and lets users observe page histories from different viewpoints. In addition, it enables flexible comparison of histories of different pages.

Modulation of neurotransmitter receptors and synaptic differentiation by proteins containing complement-related domains

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Neuroscience Research 69, 87-92 (2011.2)

Neurotransmitter receptors play central roles in basic neurotransmission and synaptic plasticity. Recent studies have revealed that some transmembrane and extracellular proteins bind to neurotransmitter receptors, forming protein complexes that are required for proper synaptic localization or gating of core receptor molecules. Consequently, the components of these complexes contribute to long-term potentiation, a process that is critical for learning and memory. Here, we review factors that regulate neurotransmitter receptors, with a focus on proteins containing CUB (complement C1r/C1s, Uegf, Bmp1) or CCP (complement control protein) domains, which are frequently found in complement system proteins. Proteins that contain these domains are structurally distinct from TARPs (transmembrane AMPA receptor regulatory proteins), and may constitute new protein families that modulate either the localization or function of neurotransmitter receptors. In addition, other CCP domain-containing proteins participate in dendritic patterning and/or synaptic differentiation, although current evidence has not identified any direct activities on neurotransmitter receptors. Some of these proteins are involved in pathologic conditions such as epileptic seizure and mental retardation. Together, these lines of information have shown that CUB and CCP domain-containing proteins contribute to a wide variety of neuronal events that ultimately establish neural circuits.

Combinatorial remarks on the cyclic sum formula for multiple zeta values

Shingo Saito, Tatsushi Tanaka* and Noriko Wakabayashi

Journal of Integer Sequences 14 (2011), no.2, Article 11.2.4, 20pp

The multiple zeta values are generalizations of the values of the Riemann zeta function at positive integers. They are known to satisfy a number of relations, among which are the cyclic sum formula. The cyclic sum formula can be stratified via linear operators defined by the second and third authors. We give the number of relations belonging to each stratum by combinatorial arguments.

Anderson-Darling test and the Malliavin calculus

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Journal of Math-for-Industry, vol.3 (2011), 73–78

The quadratic Wiener functional coming from the Anderson-Darling test statistic is investigated in the framework of the Malliavin calculus. The functional gives a completely new and important example of a quadratic Wiener functional.

Cyclic sum formula for multiple L-values

Gaku Kawashima, Tatsushi Tanaka* and Noriko Wakabayashi

Journal of Algebra 348 (2011), 336–349

The cyclic sum formula for multiple L-values, which can be viewed as a generalization of the cyclic sum formula for multiple zeta values proved by Hoffman and Ohno (or Ohno and Wakabayashi), is shown. An algebraic formulation of the cyclic sum formula is also presented.

Inflammatory Alterations of the Extracellular Matrix in the Tumor Microenvironment

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Cancers, 3(3) 3189–3205 (2011.8)

Complex interactions between cancer cells and host stromal cells result in the formation of the “tumor microenvironment”, where inflammatory alterations involve the infiltration of tumor-associated fibroblasts and inflammatory leukocytes that contribute to the acquisition of malignant characteristics, such as increased cancer cell proliferation, invasiveness, metastasis, angiogenesis, and avoidance of adaptive immunity. The microenvironment of a solid tumor is comprised not only of cellular compartments, but also of bioactive substances, including cytokines, growth factors, and extracellular matrix (ECM). ECM can act as a scaffold for cell migration, a reservoir for cytokines and growth factors, and a signal through receptor binding. During inflammation, ECM components and their degraded fragments act directly and indirectly as inflammatory stimuli in certain cases and regulate the functions of inflammatory and immune cells. One such ECM component, hyaluronan, has recently been implicated to modulate innate immune cell function through pattern recognition toll-like receptors and accelerate the recruitment and activation of tumor-associated macrophages in inflamed cancers. Here, we will summarize the molecular mechanism linking inflammation with ECM remodeling in the tumor microenvironment, with a particular emphasis on the role of hyaluronan in controlling the inflammatory response.

Telemetry system for recording neural activities in pigs-Comparison with cable system

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Brain Res Bull, 84(1) 103-9 (2011.1)

We recently developed a telemetry system for recording neural activity in the brains of unrestrained pigs. To test the fidelity of waveform reproduction, we compared local field potentials in the temporal hippocampus of six pigs by simultaneous recording with a cable system. We analyzed differences between the telemetry and cabled data filtered through a low-cut filter at 1, 4, or 30 Hz. Analysis of 10,000 data recorded while pigs were lying down showed a higher correlation with low-cut filtering at 4 or 30 Hz than at 1 Hz. Over 97% of differences in amplitude between the telemetry and cable data lay within the 95% confidence interval. Measurements were reproducible. A box plot of the differences clearly showed increased data symmetry and

reduced skewness by low-cut filtering at 4 or 30 Hz. Almost the same results were obtained in two animals during feeding. Thus, the local field potentials in the temporal hippocampus were telemetered with almost the same accuracy as by cable measurement during both resting and feeding. However, artifacts in the first 100 ms (low-cut filtering at 1 or 4 Hz) or 5 ms (30 Hz) of measurements had to be removed for analysis.

Characterization of YIPF3 and YIPF4, cis-Golgi localizing Yip domain family proteins

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Cell Structure and Function, 36, 171-185 (2011.7)

The Yip1 domain family (YIPF) proteins are homologues of yeast Yip1p and Yif1p, which are proposed to function in ER to Golgi transport. Here, we report the characterization of YIPF3 and YIPF4, homologues of human Yif1p and Yip1p, respectively. Immunofluorescence and immuno-electron microscopy showed that both YIPF3 and YIPF4 are clearly concentrated in the cis-Golgi. While YIPF4 was detected as a single mobility form consistent with its predicted molecular weight, three different mobility forms of YIPF3 were detected by western blotting. Biochemical and immunofluorescence experiments strongly indicated that YIPF3 is synthesized in the ER as a N-glycosylated form (40 kDa), is then O-glycosylated in the Golgi apparatus to become a lower mobility form (46 kDa) and finally becomes a higher mobility form cleaved at its C-terminal luminal domain (36 kDa). YIPF3 and YIPF4 form a complex in the Golgi apparatus, and this was suggested to be important for their proper localization and function. The knockdown of YIPF3 or YIPF4 in HeLa cells induced fragmentation of the Golgi apparatus, suggesting their involvement in the maintenance of the Golgi structure.

岩盤からの電磁波放射確認に向けての基礎実験

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京都産業大学先端科学研究所所報, 10, 1-14 (2011,7)

京都産業大学構内での地中電磁波観測の結果から「地震に伴い極めて短い持続時間の電磁波パルスを検出した」という事実から，その「励起機構は地球岩盤における圧電効果による」との仮説を立てた．その妥当性を検証するために，花崗岩からの電磁波放射実験を目指し，まず花崗岩に多く含まれている水晶での電磁界発生状況を明確にするために，形

状の異なる水晶への瞬間的圧力印加時での電磁界の基本的振る舞いの計測を行った．この実験では自然界での励起条件を考え，水晶には検出用電極を一切貼り付けずに電磁界を計測した．その結果，計測された電磁界の波形には様々な周期成分の振動が現れたが，それらは水晶の形状に依存している事を確認した．水晶の寸法が大きくなればその中央部で振幅の大きな電磁界が励起される事を示した．また，円柱状水晶内に生じた磁界成分の定在波から，水晶内での衝撃波の伝搬速度を求める事ができ，その速度は地震波の速度に合致している事等，詳細な電磁界励起メカニズムを明らかにできた．

Polarization and Propagation Property of Electromagnetic Pulses in the Earth

Minoru Tsutsui*, Taka Nakatani, Munetoshi Kamitani and Toshiyasu Nagao

Geoscience and Remote Sensing Symposium (IGARSS), 2011 IEEE International, 10.1109/IGARSS.2011.604926, 838-841 (2011.7)

In order to confirm the generations of electromagnetic (EM) pulses in the earth when earthquakes occurred, we have been conducting EM measurements using sensor systems composed of tri-axial magnetic search coils installed at the bottom of a 100 m-deep borehole and on the ground of a seashore. We examined polarizations of magnetic field vectors of EM pulses simultaneously detected by the sensor systems, and confirmed several kinds of propagation modes. One was vertically incident waves with linear polarization. Using their waveforms, we estimated electrical conductivity, skin-depth and phase velocity of the EM pulse in the electrically high conductive medium of the sedimentary layer around the borehole. Another is obliquely incident and ellipsoidal polarized mode, which became Zennek wave propagating along the ground surface.

Development of Poynting Vector Direction Method for Electromagnetic Pulses in the Earth

Minoru Tsutsui*, Munetoshi Kamitani and Taka Nakatani

General Assembly and Scientific Symposium, 2011 xxxth URSI 10.1109/URSIGASS.2011.6051035, 1-4 (2011.8)

We detected electromagnetic (EM) pulses in the earth whose waveforms were quite different from those generated by lightning discharges. In order to confirm their propagation directions (up- or down-ward), we conducted comparative measurements of phase and amplitude between waveforms of horizontal magnetic fields of the EM pulses detected on the ground and at the

depth of 95 m in a borehole. Clear differences in the phase and the amplitude seemed to suggest that some of them were up propagating modes and others were down propagations. We, however, found that the decision by this method was wrong, because EM pulses measured at the 95 m-depth in the borehole represented ellipsoidal polarizations whereas those simultaneously detected on the ground were linear polarizations. We concluded that EM pulses of this kind were artificial ones having propagated along the ground surface. For determining propagation directions of unknown EM pulses, we have to obtain their propagating directions from analysis of strict Poynting vector of EM pulses using field values detected by tri-axial electric dipole antennas and tri-axial magnetic search coils. In the present paper, we introduce the measuring method of Poynting vector of EM pulses in the earth.

賀茂社および摂末社の御手洗水の水質

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自然と環境，13，49-54（2011.3）

本論文では，上賀茂神社，下鴨神社およびその摂末社の御手洗水について，従来系統だった報告がないことから水質調査を行なった．いずれも，地下水をポンプで汲み上げている．今回検討した御手洗水のイオン総量の指標である電気伝導度および水質項目の濃度は，御手洗水の周りの地域に占める自然と都市地域面積の比率に影響されている場合が多かった．都市の地域面積の占める比率が増大するにつれて電気伝導度の数値が増大する傾向にあった．また，いずれの御手洗水も日本における地下水の硬度の平均 62 より低い軟水であった．

上賀茂地域の活性化を目指した住民との協働に関する研究

勝矢　淳雄*

京都産業大学総合学術研究所所報，6，21 － 38（2011.7）

本論文では，大学研究者の地域貢献の困難性を具体的な事例を基にまず論じた．その上で，社会的にはよく知られている北大路魯山人も，その出身地である上賀茂では，ほとんどの人達は上賀茂の出身であることを知らないという現状を考え，北大路魯山人生誕地石碑を上賀茂に建立するまでの住民との協働について，その経緯と問題点およびその原因と解決方法について論じた．結果として，旧小作である農家中心の自治連合会と旧支配層である社家の賀茂県主同族会が連名して石碑を建立するという上賀茂地域始まって以来の画期的な出来事となった．一方で，執拗かつ陰湿に反対した社家の某氏の行動について検討し，その根幹にある意識について考察した．今回も某氏の嫌がらせに数人の有力な人達が手を引いたが，同様のことは地域でのボランティア活動に深刻な影響を与える場合も多くあり，本論分はその対応方法を検討する上で多くの示唆を与えるものである．

伝統文化の継承・発展を目指した全国の賀茂地域の連携に関する研究

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環境衛生工学研究，25-3,144-147(2011.7)

本論文では，全国に 1700 以上ある賀茂神社および賀茂地域の連携によって地域を活性化するために全日本賀茂地域連携推進ネットワーク（賀茂ネット）を構築する必要性とその具体的な方策について論じた．一部の地域を除き，多くの賀茂地域は過疎化してきており，将来的には伝統行事の継承も困難になると考えられるが，関係者も座視しているに過ぎない状況である．また，賀茂ネットの実現のために全国の賀茂神社および賀茂地域を調査しだしているが，現在までの結果について考察し，今後の展開について論じた．

Interleukin-28B acts synergistically with cisplatin to suppress the growth of head and neck squamous cell carcinoma

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J Immunother.,34(2):139-48. 2011

Interleukin-28B (IL-28B), also referred to as interferon-λ3, belongs to the type III interferon family. Earlier studies showed that IL-28B suppresses proliferation of some tumor cells in vitro. IL-28B gene transfection ex vivo also resulted in growth retardation of tumor cells in mice, through either direct antiproliferative action or induction of antitumor immunity. However, it has not been reported whether in vivo therapeutic administration of recombinant IL-28B can inhibit the growth of a pre-established tumor. Here, we found that repetitive subcutaneous administration of recombinant mouse IL-28B significantly induced tumor-specific cytotoxic T lymphocytes and augmented natural killer cytolytic activity, leading to moderate suppression of the growth of a murine head and neck squamous cell carcinoma (HNSCC) cell line that was completely resistant to the direct antiproliferative effect of IL-28B. Moreover, co-administration of recombinant mouse IL-28B and cisplatin (CDDP) more significantly inhibited in vivo growth of the tumor that had been established in syngenic mice and induced tumor-specific cytotoxic T lymphocytes. The CDDP treatment induced the expression of major histocompatibility complex class I and Fas molecules on the surface of HNSCC cells both in vitro and in vivo; this may be the mechanism underlying the synergistic tumor suppression activity of IL-28B and CDDP. Unlike type I interferon, IL-28B did not suppress growth of bone marrow cells in culture. Therefore, IL-28B may be useful as a tool for a novel multidisciplinary therapy against cancer, significantly potentiating innate and adaptive antitumor immune responses, especially when co-administrated with CDDP, which is currently the first choice chemotherapeutic agent against various tumors including HNSCCs.

Environmental mutagens may be implicated in the emergence of drug-resistant microorganisms

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FEMS Microbiol Lett.,317(2):109-16. 2011

The emergence of drug-resistant microorganisms is an important medical and social problem. Drug-resistant microorganisms are thought to grow selectively in the presence of antibiotics. Most clinically isolated drug-resistant microorganisms have mutations in the target genes for the drugs. While any of the many mutagens in the environment may cause such genetic mutations, no reports have yet described whether these mutagens can confer drug resistance to clinically important microorganisms. We investigated how environmental mutagens might be implicated in acquired resistance to antibiotics in clinically important microorganisms, which causes human diseases. We selected mutagens found in the environment, in cigarette smoke, or in drugs, and then exposed Pseudomonas aeruginosa to them. After exposure, the incidence of rifampicin- and ciprofloxacin-resistant P. aeruginosa strains markedly increased, and we found mutations in genes for the antibiotic-target molecule. These mutations were similar to those found in drug-resistant microorganisms isolated from clinical samples. Our findings show that environmental mutagens, and an anticancer drug, are capable of inducing drug-resistant P. aeruginosa similar to strains found in clinical settings.

Jungle Honey Enhances Immune Function and Antitumor Activity

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Evidence-Based Complementary and Alternative Medicine, 2011: 1-8, 2011

Jungle honey (JH) is collected from timber and blossom by wild honey bees that live in the tropical forest of Nigeria. JH is used as a traditional medicine for colds, skin inflammation and burn wounds as well as general health care. However, the effects of JH on immune functions are not clearly known. Therefore, we investigated the effects of JH on immune functions and antitumor activity in mice. Female C57BL/6 mice were injected with JH (1 mg/mouse/day, seven times intra-peritoneal). After seven injections, peritoneal cells (PC) were obtained. Antitumor activity was assessed by growth of Lewis Lung Carcinoma/2 (LL/2) cells. PC numbers were increased in JH-injected mice compared to control mice. In Dot Plot analysis by FACS, a new cell population appeared in JH-injected mice. The percent of Gr-1 surface antigen and the intensity of Gr-1 antigen expression of PC were increased in JH-injected mice. The new

cell population was neutrophils. JH possessed chemotactic activity for neutrophils. Tumor incidence and weight were decreased in JH-injected mice. The ratio of reactive oxygen species (ROS) producing cells was increased in JH-injected mice. The effective component in JH was fractionized by gel filtration using HPLC and had an approximate molecular weight (MW) of 261. These results suggest that neutrophils induced by JH possess potent antitumor activity mediated by ROS and the effective immune component of JH is substrate of MW 261.

Inhibition of Antigen Specific and Non-specific Lymphocyte Proliferation mediated by Alveolar Macrophages in Cigarette Smoke-exposed Mice

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Acta Humanistica et Scientifica Universitatis Sangio Kyotiensis, Natural Science Series, 40: 71-108, 2011

The World Health Organization (WHO) reports that mortality from pulmonary diseases associated with exposure to cigarette smoke (CS) including respiratory infections, chronic obstructive pulmonary disease (COPD) and lung cancers has increased. It has been suggested that these diseases may be at least partially related to CS-induced impairment of the pulmonary immune system. Alveolar macrophages (AM) act as the first line of defense in the pulmonary immune system. CS reaches the lung alveoli and then directly contact with AM. However, the mechanism by which CS affects AM function is not fully understood. Therefore, we investigated the effect of CS exposure on the immunological inhibition of AM related to antigen-specific and mitogen-induced lymphocyte proliferation and whether immunological inhibition of AM would be associated with DNA damage caused by CS exposure. C57BL/6 mice were exposed to cigarette smoke for 10 days using a Hamburg II smoking machine, and AM were obtained by bronchoalveolar lavage. The antigen-presenting activity of AM was significantly inhibited in mice exposed to CS compared with mice not exposed to CS. In addition, AM from CS-exposed mice significantly inhibited B-lymphocyte proliferation stimulated with LPS compared with AM from non-CS exposed mice. Major histocompatibility complex class II cell surface molecule positive cells, B7-1 molecule positive cells, CD14 and IL-1 β mRNA gene expression in AM were significantly decreased in mice exposed to CS compared with mice not exposed to CS. In contrast, DNA damage and generation of superoxide and hydrogen peroxide in AM were significantly increased by CS exposure. Furthermore, inhibition of B-lymphocyte proliferation stimulated with LPS by AM from CS-exposed mice was clearly recovered by superoxide dismutase (SOD) and catalase. These results suggest that inhibition of the antigen-presenting activity of AM may result from decreased expression of major histocompatibility complex class II and B7-1 molecules and IL-1 β mRNA gene expression following CS exposure. Furthermore, inhibition of antigen presentation in AM may result from DNA damage induced by excessive amounts of reactive oxygen species being generated by AM following CS exposure. Our findings

suggest that CS impairs the immunological function of AM and as a result, increases the risk for pulmonary diseases.

Structural basis of an ERAD pathway mediated by the ER-resident protein disulfide reductase ERdj5

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ER-associated degradation (ERAD) is an ER quality-control process that eliminates terminally misfolded proteins. ERdj5 was recently discovered to be a key ER-resident PDI family member protein that accelerates ERAD by reducing incorrect disulfide bonds in misfolded glycoproteins recognized by EDEM1. We here solved the crystal structure of full-length ERdj5, thereby revealing that ERdj5 contains the N-terminal J domain and six tandem thioredoxin domains that can be divided into the N- and C-terminal clusters. Our systematic biochemical analyses indicated that two thioredoxin domains that constitute the C-terminal cluster form the highly reducing platform that interacts with EDEM1 and reduces EDEM1-recruited substrates, leading to their facilitated degradation. The pulse-chase experiment further provided direct evidence for the sequential movement of an ERAD substrate from calnexin to the downstream EDEM1-ERdj5 complex, and then to the retrotranslocation channel, probably through BiP. We present a detailed molecular view of how ERdj5 mediates ERAD in concert with EDEM1.

Role of heat shock protein 47 in intestinal fibrosis of experimental colitis

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Biochem Biophys Res Commun. 404(2):599-604(2011)

Background and aims: Intestinal fibrosis is a clinically important issue of inflammatory bowel disease (IBD). It is unclear whether or not heat shock protein 47 (HSP47), a collagen-specific molecular chaperone, plays a critical role in intestinal fibrosis. The aim of this study is to investigate the role of HSP47 in intestinal fibrosis of murine colitis. Methods: HSP47 expression and localization were evaluated in interleukin-10 knockout (IL-10KO) and wild-type (WT, C57BL/6) mice by immunohistochemistry. Expression of HSP47 and transforming growth factor-β1 (TGF-β1) in colonic tissue was measured. In vitro studies were conducted in NIH/3T3 cells and primary culture of myofibroblasts separated from colonic tissue of IL-10KO (PMF KO) and WT

mice (PMF WT) with stimulation of several cytokines. We evaluated the inhibitory effect of administration of small interfering RNA (siRNA) targeting HSP47 on intestinal fibrosis in IL-10KO mice in vivo. Results: Immunohistochemistry revealed HSP47 positive cells were observed in the mesenchymal and submucosal area of both WT and IL-10 KO mice. Gene expressions of HSP47 and TGF-β1 were significantly higher in IL-10KO mice than in WT mice and correlated with the severity of inflammation. In vitro experiments with NIH3T3 cells, TGF- β 1 only induced HSP47 gene expression. There was a significant difference of HSP47 gene expression between PMF KO and PMF WT. Administration of siRNA targeting HSP47 remarkably reduced collagen deposition in colonic tissue of IL-10KO mice. Conclusions: Our results indicate that HSP47 plays an essential role in intestinal fibrosis of IL-10KO mice, and may be a potential target for intestinal fibrosis associated with IBD.

SEL1L critically determines the stability of the HRD1-SEL1L ERAD complex to optimize the degradation kinetics of ERAD substrates

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The mammalian HRD1-SEL1L complex provides a scaffold for endoplasmic reticulum (ER)-associated degradation (ERAD), thereby connecting luminal substrates for ubiquitination at the cytoplasmic surface after their retrotranslocation through the endoplasmic reticulum membrane. In this study the stability of the mammalian HRD1-SEL1L complex was assessed by performing siRNA-mediated knockdown of each of its components. Although endogenous SEL1L is a long-lived protein, the half-life of SEL1L was greatly reduced when HRD1 is silenced. Conversely, transiently expressed SEL1L was rapidly degraded but was stabilized when HRD1 was coexpressed. This was in contrast to the yeast Hrd1p-Hrd3p, where Hrd1p is destabilized by the depletion of Hrd3p, the SEL1L homologue. Endogenous HRD1-SEL1L formed a large ERAD complex (Complex I) associating with numerous ERAD components including ERAD lectin OS-9, membrane-spanning Derlin-1/2, VIMP, and Herp, whereas transiently expressed HRD1-SEL1L formed a smaller complex (Complex II) that was associated with OS-9 but not with Derlin-1/2, VIMP, or Herp. Despite its lack of stable association with the latter components, Complex II supported the retrotranslocation and degradation of model ERAD substrates α 1-antitrypsin null Hong-Kong (NHK) and its variant NHK-QQQ lacking the N-glycosylation sites. NHK-QQQ was rapidly degraded when SEL1L was transiently expressed, whereas the simultaneous transfection of HRD1 diminished that effect. SEL1L unassociated with HRD1 was degraded by the ubiquitin-proteasome pathway, which suggests the involvement of a ubiquitin-ligase other than HRD1 in the rapid degradation of both SEL1L and NHK-QQQ. These results indicate that the regulation of the stability and assembly of the HRD1-SEL1L complex is critical

to optimize the degradation kinetics of ERAD substrates.

The Endoplasmic Reticulum-Associated Degradation and Disulfide Reductase ERdj5

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Methods Enzymol. 490:235-258(2011)

The endoplasmic reticulum (ER) is an organelle where secretory or membrane proteins are correctly folded with the aid of various molecular chaperones and oxidoreductases. Only correctly folded and assembled proteins are enabled to reach their final destinations, which are called as ER quality control (ERQC) mechanisms. ER-associated degradation (ERAD) is one of the ERQC mechanisms for maintaining the ER homeostasis and facilitates the elimination of misfolded or malfolded proteins accumulated in the ER. ERAD is mainly consisting of three processes: recognition of misfolded proteins for degradation in the ER, retrotranslocation of (possibly) unfolded substrates from the ER to the cytosol through dislocation channel, and their degradation in the cytosol via ubiquitin-protesome system. After briefly mentioned on productive folding of nascent polypeptides in the ER, we here overview the above three processes in ERAD system by highlighting on novel ERAD factors such as EDEM and ERdj5 in mammals and yeasts.

Hsp47 as a collagen-specific molecular chaperone

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Methods Enzymol. 499:167-182(2011)

Heat shock protein (HSP) 47 is a 47 kDa collagen-binding glycoprotein localized in the endoplasmic reticulum (ER). It belongs to the serpin family and contains a serpin loop, although it does not have serine protease inhibitory activity. The induction of Hsp47 by heat shock is regulated by a heat shock element in its promoter region, while the constitutive and tissue-specific expression of Hsp47 correlates with that of collagen and is regulated via enhancer elements located in the promoter and intron regions. Hsp47 transiently binds to procollagen in the ER and dissociates in the cis-Golgi or ER-Golgi intermediate compartment region (ERGIC). Gene ablation studies indicated that Hsp47 is essential for embryonic development and the maturation of several types of collagen. The requirement for Hsp47 in collagen maturation may reflect its ability to inhibit collagen aggregation by binding procollagen in the ER and facilitate triple helix formation. In Hsp47-deficient cells, misfolded procollagen aggregates in the ER are degraded by

the autophagy–lysosome pathway but not through the ubiquitin proteasome pathway. Hsp47 may be a therapeutic target for collagen-related disorders such as fibrosis, which feature abnormal accumulations of collagen and increased expression of Hsp47. This is supported by mouse models of fibrosis in which knockdown of Hsp47 clearly decreased the accumulation of collagen in fibrotic tissues and prevented the promotion of fibrosis. On the other hand, mutations in Hsp47 cause collagen-related genetic diseases such as osteogenesis imperfecta. Thus, Hsp47 is an indispensible molecular chaperone specific for collagen that is important in several major human diseases.

Characterization of stress sensitivity and chaperone activity of Hsp105 in mammalian cells

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Biochem Biophys Res Commun. 409:90-95(2011)

Hsp105 is a major mammalian heat shock protein that belongs to the Hsp105/110 family, a diverged subgroup of the Hsp70 family. Hsp105 not only protects the thermal aggregation of proteins, but also regulates the Hsc70 chaperone system in vitro. Recently, it has been shown that Hsp105/110 family members act as nucleotide exchange factors for cytosolic Hsp70s. However, the biological functions of Hsp105/110 family proteins still remain to be clarified. Here, we examined the function of Hsp105 in mammalian cells, and showed that the sensitivity to various stresses was enhanced in the Hsp105-deficient cells compared with that in control cells. In addition, we found that deficiency of Hsp105 impaired the refolding of heat-denatured luciferase in mammalian cells. In contrast, overexpression of Hsp105a enhanced the ability to recover heat-inactivated luciferase in mammalian cells. Thus, Hsp105 may play an important role in the refolding of denatured proteins and protection against stress-induced cell death in mammalian cells.

Protein Folding and Quality Control in the ER

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The endoplasmic reticulum (ER) uses an elaborate surveillance system called the ER quality control (ERQC) system. The ERQC facilitates folding and modification of secretory and membrane proteins and eliminates terminally misfolded polypeptides through ER-associated degradation (ERAD) or autophagic degradation. This mechanism of ER protein surveillance

is closely linked to redox and calcium homeostasis in the ER, whose balance is presumed to be regulated by a specific cellular compartment. The potential to modulate proteostasis and metabolism with chemical compounds or targeted siRNAs may offer an ideal option for the treatment of disease.

Identification of RNF213 as a Susceptibility Gene for Moyamoya Disease and Its Possible Role in Vascular Development

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BACKGROUND: Moyamoya disease is an idiopathic vascular disorder of intracranial arteries. Its susceptibility locus has been mapped to 17q25.3 in Japanese families, but the susceptibility gene is unknown.

METHODOLOGY/PRINCIPAL FINDINGS: Genome-wide linkage analysis in eight three-generation families with moyamoya disease revealed linkage to 17q25.3 ($P<10(-4)$). Fine mapping demonstrated a 1.5-Mb disease locus bounded by D17S1806 and rs2280147. We conducted exome analysis of the eight index cases in these families, with results filtered through Ng criteria. There was a variant of p.N321S in PCMTD1 and p.R4810K in RNF213 in the 1.5-Mb locus of the eight index cases. The p.N321S variant in PCMTD1 could not be confirmed by the Sanger method. Sequencing RNF213 in 42 index cases confirmed p.R4810K and revealed it to be the only unregistered variant. Genotyping 39 SNPs around RNF213 revealed a founder haplotype transmitted in 42 families. Sequencing the 260-kb region covering the founder haplotype in one index case did not show any coding variants except p.R4810K. A case-control study demonstrated strong association of p.R4810K with moyamoya disease in East Asian populations (251 cases and 707 controls) with an odds ratio of 111.8 ($P=10(-119)$). Sequencing of RNF213 in East Asian cases revealed additional novel variants: p.D4863N, p.E4950D, p.A5021V, p.D5160E, and p.E5176G. Among Caucasian cases, variants p.N3962D, p.D4013N, p.R4062Q and p.P4608S were identified. RNF213 encodes a 591-kDa cytosolic protein that possesses two functional domains: a Walker motif and a RING finger domain. These exhibit ATPase and ubiquitin ligase activities. Although the mutant alleles (p.R4810K or p.D4013N in the RING domain) did not affect transcription levels or ubiquitination activity, knockdown of RNF213 in zebrafish caused irregular wall formation in trunk arteries and abnormal sprouting vessels.

CONCLUSIONS/SIGNIFICANCE: We provide evidence suggesting, for the first time, the involvement of RNF213 in genetic susceptibility to moyamoya disease.

Functional in vitro analysis of ERO1 and protein-disulfide isomerase (PDI) pathway

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Oxidative protein folding in the endoplasmic reticulum is supported by efficient electron relays driven by enzymatic reactions centering on the ERO1-protein-disulfide isomerase (PDI) pathway. A controlled in vitro oxygen consumption assay was carried out to analyze the ERO1-PDI reaction. The results showed the pH-dependent oxidation of PDI by ERO1 α. Among several possible disulfide bonds regulating ERO1 α activity, Cys^{94}-Cys131 and Cys^{99}-Cys^{104} disulfide bonds are dominant regulators by excluding the involvement of the Cys^{85}-Cys^{391} disulfide in the regulation. The fine-tuned species specificity of the ERO1-PDI pathway was demonstrated by functional in vitro complementation assays using yeast and mammalian oxidoreductases. Finally, the results provide experimental evidence for the intramolecular electron transfer from the a domain to the a' domain within PDI during its oxidation by ERO1α.

Analyzing the aggregation of polyglutamine-expansion proteins and its modulation by molecular chaperones

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Methods 53(3):267-274(2011)

Polyglutamine (polyQ)-expansion proteins cause protein aggregation in the cytosol and nucleus of neuronal cells, leading to neurodegenerative diseases. For example, expansion of the polyQ tract (>40 repeats) in huntingtin (htt) proteins leads to Huntington disease, while polyQ-expanded ataxins cause several types of ataxias. PolyQ-rich inclusions are found in neuronal cells of patients, suggesting that polyQ disease is caused by protein misfolding. However, the mechanisms by which polyQ-expansion proteins exert neuronal toxicity are largely unknown. Here, we review experimental procedures to analyze the roles of molecular chaperones in preventing polyQ aggregation and toxicity as well as to measure the characteristics and dynamics of polyQ aggregation, particularly focusing on cellular models and dynamic imaging of fluorescently-labeled polyQ-expansion proteins and their modulation by chaperones.

Type A1 but Not Type A2 Botulinum Toxin Decreases the Grip Strength of the Contralateral Foreleg Through Axonal Transport From the Toxin-Treated Foreleg of Rats

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J Pharmacol Sci. 117:275-285 (2011.11)

The adverse effects of botulinum LL toxin and neurotoxin produced by subtype A1 (A1LL and A1NTX) are becoming issues, as the toxins could diffuse from the toxin-treated (ipsilateral) to contralateral muscles. We have attempted to produce neurotoxin from subtype A2 (A2NTX) with an amino acid sequence different from that of neurotoxin subtype A1. We measured the grip strength on the contralateral foreleg as an indicator of toxin spread from the ipsilateral to contralateral muscles. Doses of 0.30 log U or above of A1LL and A1NTX reduced the contralateral grip strength, whereas a dose of 0.78 log U of A2NTX was required to do so. We investigated the route of toxin spread using denervated, colchicine-treated, and antitoxin-treated rats. A1LL was transported via axons at doses higher than 0.30 log U and via both axons and body fluid at about 0.80 log U or a higher dose. Interestingly, A2NTX was transported via body fluid at about 0.80 log U or a higher dose, but not via axons to the contralateral side. It was concluded that A1LL and A1NTX decreased the grip strength of the toxin-untreated foreleg via both axonal transport and body fluids, while A2NTX was only transported via the body fluid.

Introduction of a novel molecular mechanism of epilepsy progression: roles of growth hormone signaling in a mouse model of temporal lobe epilepsy

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About one-half of patients with refractory epilepsy are characterized as having mesial temporal lobe epilepsy with foci in the amygdaloid complex, hippocampus, and surrounding cortex. To screen candidate molecules that be involved in refractory epilepsy, we have used amygdala-kindled mice, a model of temporal lobe epilepsy, in which unrestrained conscious mice received a biphasic square wave pulse [480 micro-A; 60 Hz, 200 micro-sec duration, for 2 sec] once a day for about 3 weeks. We found that the expression of growth hormone was up-regulated along neural circuits during epileptogenesis and exogenous growth hormone enhanced the progression of

epilepsy. Moreover, exogenous growth hormone without kindling-stimulation caused increases of seizure-responsive gene expressions, which suggests that signaling pathway via growth hormone exists in brain and the up-regulation is involved in epileptogenesis. In the peripheral organs, it is known that growth hormone is related with lipid metabolism. We have also observed up-regulation of GQ1b in the hippocampus following kindled-seizures, showing that GH signaling in brain might act up-regulation of GQ1b on epilepsy progression. The present review summarizes a distinctive key system to propagate epileptic-stimuli by growth hormone signaling.