様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　２ 年　 ６ 月 １１ 日現在

機関番号：２２７０１
研究種目：基盤研究(C)（一般）
研究期間：2017～2019
課題番号：１７Ｋ００５５５

研究課題名（和文）Establishment of DNA adductomics to study human cells

研究課題名（英文）Establishment of DNA adductomics to study human cells

研究代表者
Ｒｏｂｅｒｔ　Ｋａｎａｌｙ（Kanaly, Robert）

横浜市立大学・理学部・教授

研究者番号：００４４８６５６

交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：トリプル四重極型質量分析を用いた比較アダクトミクス法による、有毒な芳香族化合物に曝露されたヒト細胞におけるDNA/RNA付加体を検出する実験パイプラインを構築した。核酸アダクトミクス解析に高分解能質量分析とNMR分析を組み合わせ、食品成分、環境汚染物質、大気汚染物質、およびアリルヒドロキシルアミンの曝露によって生じる新規・既知の核酸付加体の検出と化学構造の特定に成功し、また、それらの生成機序について提案した。このDNA/RNAアダクトミクス法をDNA毒性・変異原性試験に応用することで、従来のDNAアダクトミクス法で生じる偽陽性を排除できる新たな毒性評価法の発展に繋がることが期待される。

研究成果の学術的意義や社会的意義
Results of this work contributed to the advancement of the fields of nucleic acid adductomics and DNA damage research with applicability to human exposure and drug development. A new methodology, DNA/RNA adductomics was designed, tested and its proof-of-concept was demonstrated for the first time.

研究成果の概要（英文）：DNA and DNA/RNA adduct screening pipelines were constructed to conduct triple quadrupole mass spectrometry comparative adductomics by liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI(+)-MS/MS) utilizing human cell lines that were exposed to hazardous aromatic chemicals. Nucleic acid adductomics approaches were combined with high resolution-(HR-MS) and NMR analyses to detect and to identify adducts of the naturally-occurring food constituent, safrole, the environmental pollutant, benzo[a]pyrene, the air pollutant, 1, 2-naphthoquinone, and the arylhydroxylamine, N-(2,6-dimethylphenyl)hydroxylamine. The chemical structures of novel and known adducts and their mechanisms of formation were proposed and a new methodology for verification of DNA adductomics results to eliminate false positive annotations when it is applied as a DNA damage/genotoxicity screening test was developed and demonstrated by utilization of both DNA and RNA adductomics pipelines.

研究分野：環境毒性学

キーワード：DNA adductomics　RNA adductomics　DNA damage　1,2-Naphthoquinone　Arylamines

※科研費による研究は、研究者の自覚と責任において実施するものです。そのため、研究の実施や研究成果の公表等については、国の要請等に基づくものではなく、その研究成果に関する見解や責任は、研究者個人に帰属されます。

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Interactions between DNA and reactive chemicals in living cells may lead to structural modifications called DNA adducts. DNA adducts may be considered to be a form of DNA damage that results in harmful effects to cells and they may have roles in chemically-induced carcinogenesis in living organisms. Currently, there are gaps in our understanding of the types of DNA adducts that may occur in cells and their potential downstream effects. The formation of DNA adducts and downstream effects occur by complex mechanisms that depend upon many factors including cell type, DNA repair processes and exposure conditions.

Interest in understanding DNA damage that arises from exogenous exposures to small and large molecule aromatic chemicals through occupation, diet, and other less-defined routes is increasing as more information about the potential hazards of these chemicals becomes known. Due to the dynamic nature of the occurrence of DNA adducts and the complexity of mechanisms involved, innovative approaches to study DNA adducts are necessary.

２．研究の目的

To investigate DNA adduct formation of aromatic chemicals by using liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) and liquid chromatography electrospray ionization high resolution mass spectrometry (LC/ESI-HR/MS) comparative adductomics approaches and to establish pipelines for the application of DNA adductomics to human cell lines.

３．研究の方法

Human cell lines were exposed to different classes of aromatic compounds after which cells were extracted for DNA or RNA. After extraction, nucleic acids were digested by different techniques by enzyme and heat treatment. DNA was hydrolyzed by micrococcal nuclease and spleen phosphodiesterase and digested at 37^{o}C. Digestion was completed by incubating the samples with alkaline phosphatase at 37^{o}C and methanol extraction. RNA was treated identically to DNA in the first step after which it was digested with RNase A, nuclease P1, and alkaline phosphatase at 37^{o}C and extracted into methanol. Following sample preparation, digested 2'-deoxynucleosides and ribonucleosides were analyzed by LC/ESI(+)-MS/MS and LC/ESI(+)-HR/MS using various mass spectrometry techniques and nucleic acid adductomics methods were applied to evaluate the results. 2’-deoxyguanosine was exposed to different types of aromatic compounds and the products were analyzed by LC/ESI(+/-)-MS/MS, LC/ESI(+)-HR/MS, and different types of NMR techniques.

４．研究成果

Comparative DNA adductomics analyses of human hepatoma (HepG2) cells exposed to the naturally-occurring plant constituent, safrole (4-allyl-1,2-methylenedioxybenzene) showed that numerous putative DNA adduct candidates occurred in exposed cells. Chemical structures for five of the DNA adduct candidates were proposed based upon different analytical approaches and a biotransformation pathway to describe their formation in human cells was constructed and proposed.

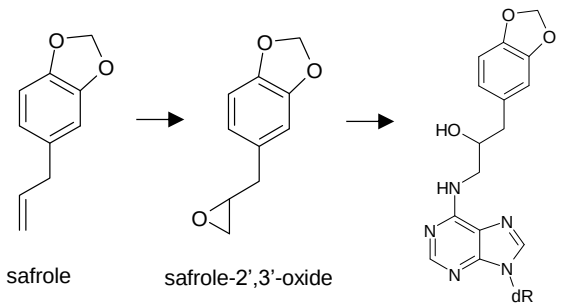

Fig. 1 Formation of a DNA adduct detected in HepG2 cells caused by exposure to safrole that was detected by comparative DNA adductomics.

Comparative DNA/RNA adductomics analyses were applied independently as a methodology to confirm putative DNA adducts that were originally detected by DNA adductomics by utilizing the HepG2 cell line and the polycyclic aromatic environmental pollutant, benzo[*a*]pyrene. Analogous forms of nucleic acid damage were detected by LC/ESI(+)-MS/MS and the identities of the DNA and RNA adducts were confirmed by LC/ESI(+)-HR/MS techniques. Overall, *in vitro* DNA adductome methods in conjunction with *in vitro* RNA adductome methods, *i.e.*, DNA/RNA adductomics, was proposed as a new methodology for verification of DNA adductome results by which to eliminate false positive annotations when DNA adductomics is applied as a DNA damage/genotoxicity screening test.

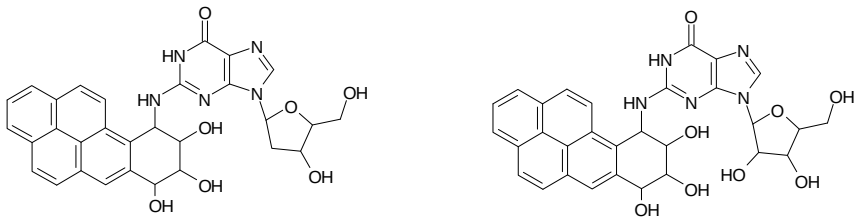

Fig. 2 Benzo[*a*]pyrene adducts of DNA and RNA that were detected by comparative DNA/RNA adductomics analyses utilizing both LC/ESI(+)-MS/MS and LC/ESI(+)-HR/MS techniques.

LC/ESI(+/-)-MS/MS and ^{1}H NMR analyses of adduct formation caused by exposure of 2'-deoxyguanosine to the aromatic pollutant, 1,2-naphthoquinone were conducted and results supported that 1,2-naphthoquinone was non-enzymatically oxidized to become 1,2-naphthoquinone-epoxide which reacted with 2'-deoxyguanosine to form at least four bulky 2'-deoxyguanosine adducts. A mechanism was constructed and proposed that involved initiation of the reaction process through hydration of 1,2-naphthoquinone to form unstable naphthohydroquinones and 2-hydroxy-1,4-naphthoquinone that resulted in the production of hydrogen peroxide. Hydrogen peroxide in turn oxidized 1,2-naphthoquinone to reactive 1,2-naphthoquinone-epoxide which in turn reacted with 2'-deoxyguanosine.

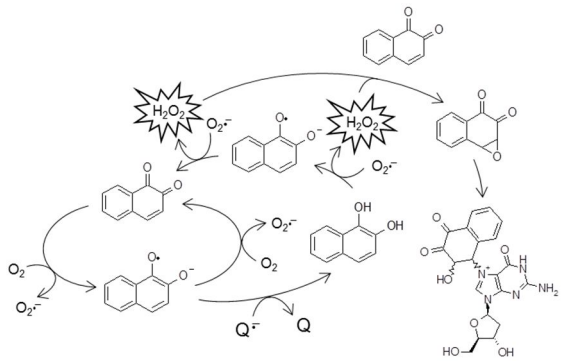

Fig. 3 Simplified mechanism describing production of a bulky adduct through 1,2-naphthoquinone oxidation.

When 2'-deoxyguanosine was exposed to *N*-(2,6-dimethylphenyl)hydroxylamine (2,6-DMPHA) and *N*-phenylhydroxylamine (PHA) under weakly basic conditions and analyzed by LC/ESI(+)-HRMS and various NMR techniques at least six different types of bulky adducts and their corresponding structures were proposed based upon comprehensive MS spectral and NMR analyses. Adducts that formed without conjugation through different positions on 2'-deoxyguanosine were identified and mechanisms for the formation of these adducts were constructed and proposed.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　1件）

１．著者名 Matsui, T., N. Yamada, H. Kuno and R.A. Kanaly	４．巻 252
２．論文標題 Characterization of N-(2,6-dimethylphenyl)hydroxylamine adducts of 2′-deoxyguanosine under weakly basic conditions	５．発行年 2020年
３．雑誌名 Chemosphere	６．最初と最後の頁 126530
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.chemosphere.2020.126530	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Maeda, A.H., S. Nishi, Y. Hatada, Y. Ohta, K. Misaka, M. Kunihiro, J.F. Mori and R.A. Kanaly	４．巻 151
２．論文標題 Chemical and genomic analyses of polycyclic aromatic hydrocarbon biodegradation in Sphingobium barthaii KK22 reveals divergent pathways in soil sphingomonads	５．発行年 2020年
３．雑誌名 International Biodeterioration & Biodegradation	６．最初と最後の頁 104993
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.ibiod.2020.104993	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Matsui, T., N. Yamada, H. Kuno and R.A. Kanaly	４．巻 32
２．論文標題 Formation of bulky DNA adducts by non-enzymatic production of 1,2-naphthoquinone-epoxide from 1,2-naphthoquinone under physiological conditions	５．発行年 2019年
３．雑誌名 Chemical Research in Toxicology	６．最初と最後の頁 1760-1771
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.chemrestox.9b00088	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Takeshita, T., and R.A. Kanaly	４．巻 7
２．論文標題 In vitro DNA/RNA adductomics to confirm DNA damage caused by benzo[a]pyrene in the Hep G2 cell line.	５．発行年 2019年
３．雑誌名 Frontiers in Chemistry	６．最初と最後の頁 491
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fchem.2019.00491	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Takeshita, T., F. Tao, N. Kojima and R.A. Kanaly	4．巻 300
2．論文標題 Triple quadrupole mass spectrometry comparative DNA adductomics of Hep G2 cells following exposure to safrole	5．発行年 2019年
3．雑誌名 Toxicology Letters	6．最初と最後の頁 92-104
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.toxlet.2018.10.023	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計14件（うち招待講演　0件／うち国際学会　10件）

1．発表者名 Matsui, T., N. Yamada, H. Kuno and R.A. Kanaly
2．発表標題 Investigation of an adduct formation mechanism for N-oxidized aniline derivatives with 2’-deoxyguanosine
3．学会等名 The 37th Medicinal Chemistry Symposium (MSC2019)
4．発表年 2019年

1．発表者名 Takeshita, T., H. Sakagami, M. Tachikawa and R.A. Kanaly
2．発表標題 Verification of DNA adduct formation derived from chemical compounds by DNA/RNA adductome methods and in silico: ab initio calculation analyses
3．学会等名 Joint Meeting of the 6th Asian Congress on Environmental Mutagens (ACEM) and the 48th Annual Meeting of the Japanese Environmental Mutagen Society (JEMS)（国際学会）
4．発表年 2019年

1．発表者名 Yamazeki, Y., R. Kanauchi, A. Nagano, R.A. Kanaly and T. Takeshita
2．発表標題 Examination of the micronucleus test in T24 human bladder carcinoma cells
3．学会等名 Joint Meeting of the 6th Asian Congress on Environmental Mutagens (ACEM) and the 48th Annual Meeting of the Japanese Environmental Mutagen Society (JEMS)（国際学会）
4．発表年 2019年

１．発表者名
Matsumoto, A., R. Koga, R.A. Kanaly, A. Kouzuma and K. Watanabe

２．発表標題
SO_1646 encodes a diguanylate cyclase that determines intracellular cyclic di-GMP levels and regulates biofilm formation and current generation by Shewenella oneidensis MR-1

３．学会等名
International Society for Microbial Electrochemistry and Technology 7 (ISMET 7 2019)（国際学会）

４．発表年
2019年

１．発表者名
Mori, J.F., Y. Tomiyama and R.A. Kanaly

２．発表標題
The oil-eating gangs: metagenomics revealed the multi-species oil hydrocarbon-degrading soil bacterial consortium evolved in the lab-scale enrichment

３．学会等名
The 33rd Annual Meeting of the Japanese Society of Microbial Ecology (JSME)（国際学会）

４．発表年
2019年

１．発表者名
Tomiyama, Y., J.F. Mori and R.A. Kanaly

２．発表標題
Investigation of a hydrocarbon pollutant-degrading bacterial consortium and Bradyrhizobium sp. strain KK5

３．学会等名
The 33rd Annual Meeting of the Japanese Society of Microbial Ecology (JSME)（国際学会）

４．発表年
2019年

１．発表者名
Takeshita, T., and R.A. Kanaly

２．発表標題
Comparative investigation of DNA and RNA adductome methods to detect nucleic acid-reactive metabolites

３．学会等名
The 45th Annual Meeting of the Japanese Society of Toxicology（国際学会）

４．発表年
2018年

１．発表者名
Takeshita, T., and R.A. Kanaly

２．発表標題
Comparative non-targeted DNA adductomics of Hep G2 cells exposed to substituted monaromatic compounds

３．学会等名
Society of Environmental Toxicology and Chemistry Asia-Pacific 2018（国際学会）

４．発表年
2018年

１．発表者名
Matsumoto, A., Koga, R., Kanaly, R.A., Kouzuma, A., and K. Watanabe

２．発表標題
Involvement of c-di-GMP in power generation during biofilm formation

３．学会等名
Kanto Branch Meeting of the Japan Society for Bioscience, Biotechnology and Biochemistry (JSBBA)

４．発表年
2018年

１．発表者名
Takeshita, T., Takaki, Y., Murayama, K., and R.A. Kanaly

２．発表標題
RNA transcriptomics and DNA adduct analyses to investigate the effects of naphthoquinone on bacterial cells

３．学会等名
47th Annual Meeting of the Japanese Environmental Mutagen Society（国際学会）

４．発表年
2018年

１．発表者名
Takeshita, T., and R.A. Kanaly

２．発表標題
Application of the DNA/RNA adductome method for nucleic acid injury investigation of thiazolidinedione compounds

３．学会等名
47th Annual Meeting of the Japanese Environmental Mutagen Society（国際学会）

４．発表年
2018年

１．発表者名
Takeshita, T., F. Tao, N. Kojima and R.A. Kanaly

２．発表標題
Comprehensive analysis of DNA adducts in human liver cells after exposure to safrole

３．学会等名
The 23st General Meeting of the Japanese Society of Food Chemistry

４．発表年
2017年

１．発表者名
Takeshita, T., F. Tao, N. Kojima and R.A. Kanaly

２．発表標題
Investigation of reactive metabolites in human liver cell line by use of the DNA adductome method

３．学会等名
Consortium of Biological Sciences 2017 (ConBio2017)（国際学会）

４．発表年
2017年

１．発表者名
Takeshita, T., and R.A. Kanaly

２．発表標題
Investigation of intracellular RNA damage to improve chemical safety assessment methods

３．学会等名
The Seventh Intercollegiate Conference on Science in Japan, Japan Ministry of Education, Culture, Sports, Science and Technology

４．発表年
2017年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考