m^6 RNA methylation: an emerging common target in the immune response to cancer and severe acute respiratory syndrome-coronavirus-2 infection

Hiromichi Sato[1,2] , Tomoaki Hara[1], Chihiro Otsuka[1], Yasuko Arao[1], Yoshiko Tsuji[1], Yumiko Hamano[1], Mirei Ogita[1], Eric di Luccio[3], Takaaki Hirotsu[3], Andrea Vecchione[4], Hideshi Ishii[1]*

[1]Department of Medical Data Science, Center of Medical Innovation and Translational Research, Osaka University Graduate School of Medicine, Suita, Yamadaoka 2-2, Osaka 565-0871, Japan

[2]Department of Gastrointestinal Surgery, Osaka University Graduate School of Medicine, Suita, Yamadaoka 2-2, Osaka 565-0871, Japan

[3]Hirotsu Bio Science Inc., Chiyoda-Ku, Tokyo 102-0094, Japan

[4]Department of Clinical and Molecular Medicine, Sapienza University of Rome, Santo Andrea Hospital, Via di Grottarossa, 1035-00189 Rome, Italy

*Correspondence: Hideshi Ishii, Department of Medical Data Science, Center of Medical Innovation and Translational Research, Osaka University Graduate School of Medicine, Suita, Yamadaoka 2-2, Osaka, 565-0871, Japan. hishii@gesurg.med.osaka-u.ac.jp

Abstract

m^{6}A RNA methylation, a predominant type of RNA modification, is involved in regulating mRNA splicing, stability, and translation as well as the interaction between nucleoproteins and noncoding RNAs. Recent studies have revealed that m^{6}A RNA methylation plays a critical role in the self-to-non-self-recognition of immune cells against endogenous mutations in cancer and exogenous organism-related infections. As an epigenetic mechanism, m^{6}A RNA modification induces immune cell signal transduction, which is altered in the tumor microenvironment, as detected in liquid biopsy. Furthermore, m^{6}A RNA methylation-related inflammation is involved in the cellular response to viral infections, including the emerging severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection. Given the importance of the immune response in maintaining homeostasis in higher eukaryotes, m^{6}A RNA methylation could be useful not only for the early detection of cancer but also for SARS-CoV-2 screening during a global pandemic.

Keywords

RNA, methylation, m^{6}A, cancer, severe acute respiratory syndrome-coronavirus-2

Introduction

Recently, RNA modification has emerged as a critical epigenetic mechanism involved in the regulation of embryonic stem cell development [1], immune cell response [2], and various conditions, such as infection [3]

and cancer progression and development [4]. As a mechanism, RNA modification, the most common and critical in function and stability of which is N^6-methylation, is reported to affect folding, stability, degradation, and multiple processes, such as splicing, peptide translation, export, and decay [5]. Furthermore, RNA modification is involved in cellular interactions [5]. The mechanism has been elucidated via the structural characterization of RNA methyltransferases (MTs) [5]. RNA methylation is characterized by three steps that are similar to those of histone modification, namely, writing, erasing, and reading. Thus, the dynamic and reversible chemical m⁶A RNA methylation may serve as a critical epigenetic marker of biological significance given the pivotal role of DNA and histone methylation in basic life processes [6]. MT-like 3 (METTL3; N^6-adenosine-MT complex catalytic subunit) and METTL14 (N^6-adenosine-MT subunit) form a stable heterodimeric core complex that functions in cellular m⁶A deposition on nuclear RNAs and catalyzes m⁶A RNA methylation [7]. Wilms' tumor 1-associating protein (WTAP), a mammalian splicing factor, can interact with this complex and influence its methylation [7]. The core complex of METTL3–14 with WTAP induces methylation at N^6-adenosines [8, 9]. Functional demethylases that reverse this methylation have been identified as erasers, including fat mass and obesity-associated (FTO) protein [10] and alpha-ketoglutarate-dependent dioxygenase AlkB homolog 5 (ALKBH5) [11]. However, several factors have been reported as readers, including heterogeneous nuclear ribonucleoprotein (hnRNP) [12] and YTH N^6-methyladenosine RNA-binding proteins [YTH domain family (YTHDF)] [13]. Previous studies have indicated that m⁶A RNA methylation provides diagnostic and therapeutic targets for innovative medicine against human diseases such as cancer [14]. Nonetheless, recent studies have shown that m⁶A RNA methylation may also give rise to potential targets for the treatment of infectious diseases caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) [15], a highly transmissible coronavirus that emerged in late 2019 and is now spreading globally [16]. This concept has several key implications. First, as a scientific implication, the biological response to exogenous and endogenous nucleic acids involves a shared mechanism of RNA methylation, which is one of the processes of biological homeostasis maintenance. Second, drugs developed for cancer treatment can be used to treat other diseases via repositioning, thereby significantly reducing the cost and time required for therapy development. Third, it is expected that if the mechanisms shared by multiple diseases can be used in diagnosis, social implementation in medical and health examinations could be accelerated.

Cellular response to RNA methylation

The process of pathogen elimination by immunological activation includes rapidly sensing the invasion of pathogenic microorganisms. Hence, previous research on the sensing mechanism of biomaterials and many other signaling patterns has focused on toll-like receptors (TLRs), which has allowed the discovery of many pattern recognition receptors (PRRs) [17]. PRRs provide the structures of various constituent molecules derived from microorganisms, such as pathogen-associated molecular patterns, which consist of sugars, lipids, proteins, and nucleic acids [18]. In humans, PRRs have been reported to play a role in infections and also serve as sensors for internal abnormalities, such as biological processes of tissue damage and repair caused by stress or cancer [17]. Therefore, in response to dangerous conditions in an organism, the biological defense system is activated to quickly sense the hazard and emit a "danger signal," thus maintaining homeostasis [19]. Previous research has shown that nucleic acid sensing mechanisms play critical roles in biological defense systems [20]. Compared with DNA recognition, the RNA recognition mechanism in the innate immune system is well characterized. Several RNA sensor molecules have been identified as their downstream signals, including cell membrane binding TLR3, TLR7, and TLR8 [21, 22] as well as cytoplasmic antiviral innate immune response receptors, such as retinoic acid-inducible gene I (*RIG-I*)'s product, interferon (IFN) induced with helicase C domain 1 such as melanoma differentiation-associated protein 5 (MDA5), and *RIG-I*-like receptor such as ATP-dependent helicase (LGP2) [23–26]. *RIG-I* is essential for RNA sensing, thus triggering antiviral and inflammatory responses that control RNA viral replication in response to virus-specific RNA structures [27]. Upon viral RNA recognition, *RIG-I* has been reported to recruit the adaptor protein, a mitochondrial antiviral signaling protein, on the outer mitochondrial membrane. This recruitment leads to a signaling cascade of type I IFNs [27]. *RIG-I* activation

is tightly regulated via various post-translational modifications to prevent aberrant innate immune signaling. However, viruses have evolved evasion mechanisms, such as sequestering viral structures from *RIG-I* detections, which can target receptor or signaling molecules for degradation [27]. A previous study has demonstrated that *RIG-I* triggers an antiviral response upon binding non-self RNA with a stretch of double-stranded RNA bearing a base-paired 5'ppp nucleotide [28]. *RIG-I* adenosine triphosphatase activity can restrict the interaction between *RIG-I* signaling and non-self RNA [28]. Importantly, research on RNA modification has revealed that viruses acquire m^{6}A in their RNA as a means of mimicking cellular RNA to avoid detection by innate immunity. This finding implies that the m^{6}A modification enables viral RNA to evade RNA sensor *RIG-I* [29]. A study has suggested that viral m^{6}A RNA modification can be used as a vaccine target to attenuate viral infections, such as those caused by human metapneumovirus [29]. Furthermore, a recent investigation has observed that METTL3 regulates viral m^{6}A RNA modification and host cell innate immune responses during SARS-CoV-2 infection [30]. The study also reported that the SARS-CoV-2 genome contains m^{6}A modifications enriched in the 3' region of RNA and that *RIG-I* binds to SARS-CoV-2 viral RNAs and is inhibited by m^{6}A RNA modifications [30]. In patients with severe coronavirus disease 2019 (COVID-19), METTL3 is reduced and inflammatory genes are induced [30], which agrees with the findings in other viruses [29]. It has been shown that host m^{6}A components interact with viral proteins to modulate SARS-CoV-2 replication [31]. It has further been suggested that SARS-CoV-2 can cause an imbalance of methylated and unmethylated RNA via METTL3. This phenomenon may be involved in host recognition as well as in eliciting a severe immune response and the resultant adverse effects in patients with COVID-19. Further research is thus needed on the relationship between METTL3 activity and m^{6}A modification status and biological response in patients with COVID-19.

RNA methylation therapy against cancer

The role of RNA methylation in cancer has been investigated [4]. Recent studies have revealed that RNA methylation is involved in the biological behaviors of glioblastoma stem cells [32], self-renewal of leukemic stem cells [33], therapeutic implications of coding and noncoding RNAs [34], and direct link between cancer metabolism and progression [35]. The m^{6}A modification is associated not only with cancer but also with cardiovascular diseases [36] and Alzheimer's disease [37]. Recent studies have revealed that small-molecule (STM2457) inhibition of METTL3 can serve as a favorable strategy against myeloid leukemia [38, 39]. Eltrombopag, a nonpeptide agonist of the active thrombopoietin receptor encoded by the myeloproliferative leukemia virus proto-oncogene (c-Mpl), is used to treat thrombocytopenia and has recently been reported as a selective allosteric inhibitor of the METTL3–14 complex [40].

RNA methylation in cancer detection and COVID-19 monitoring

The study of RNA methylation in microRNAs (miRNAs) and short noncoding RNAs revealed that RNA methylation at adenine and cytosine is relatively specific in gastrointestinal cancers, such as pancreatic cancer, as detected with mass spectrometry in serum via liquid biopsy and in surgically resected samples via solid biopsy [41]. Tunnel current sequencing analysis revealed that miRNAs derived from cancer cells had varying fingerprints in adenine and cytosine [42]. As exosomal miRNAs in liquid biopsy can be derived from tumor tissues as a whole-body response, it has been suggested that the profiling of RNA modification provides useful information not only for the early detection of cancer but also for determining the responses to chemotherapy, radiotherapy, and immunotherapy. Nonetheless, which methods are suitable for the diagnosis or monitoring of cancers in the pancreas [43], stomach [44], lung [45], colon, and rectum [46] is yet to be ascertained.

Recent studies have revealed that a volatile organic compound (VOC)–ozone mixture has a functional impact on RNA chemistry, including the prominent RNA oxidative modification 8-oxo-7,8-dihydroguanine, in lung epithelial cells. This finding offers a new insight into the relationship between atmospheric agents and cellular responses involved in human diseases [47], as being more consistent markers of cellular stress relative to other inflammation markers, such as interleukin 8 and lactate dehydrogenase [48]. In this regard, attempts have recently been made to utilize VOC-based methods for cancer diagnosis [49, 50]. Although

several odorants have been detected with mass spectrometry, animal-based detection methods are capable of detecting multiple or complex signaling responses. Thus, such methods have the potential to pave the way for new modalities [51]. Although previous studies have demonstrated the efficacy of a low-cost cancer screening test using cancer-sniffing canines, a more affordable and accessible option is desired, especially for low-resourced populations [52]. A recent study found that a scent test using nematodes is effective in screening for early-stage pancreatic cancer [53]. These results indicate that the methods should be studied further for the detection of refractory cancers.

It has been shown that smell dysfunction to some extent is associated with COVID-19 in > 90% of the cases [54], a recent study found that non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system is likely to be involved in the mechanisms underlying COVID-19-associated anosmia [55]. The exact mechanism in infected cells, i.e., viral RNA-mediated activation of PRRs or involvement of *RIG-1* and MDA5, remains unknown (Figure 1).
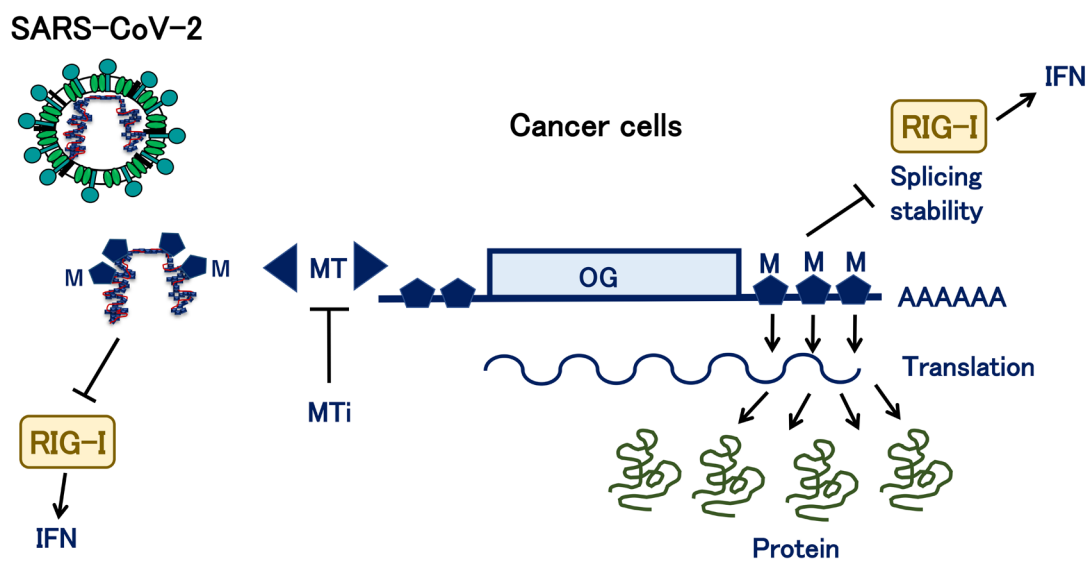

Figure 1. A schematic representation of the parallel involvement of RNA MT in cancer and COVID-19. OG: oncogene; MTi: MT inhibitors; M: methylated residue; A: multiple adenosine monophosphates in poly (A) tail

Discussion

Although RNA modifications include many changes to the chemical composition of RNA molecules post-synthesis that have the potential to alter function or stability, previous studies indicated that methylation of RNA affects heterogeneity of malignant tumors [4]. Among the various RNA methylations, m^6A RNA modifications has been characterized well as therapeutic targets [4]. Here we noted that a repositioning of m^6A RNA targeting from cancers to infectious diseases may be possible. At first glance, cancer and COVID-19 appear unrelated, but they are entwined like a rope. First, as the common mechanism, RNA modification, which is important in cancer, plays a key role in the recognition of SARS-CoV-2 by the host cells and in the biological response to the infection. Second, epoch-making diagnostic and drug discovery technologies targeting RNA modifications, especially RNA N^6-methylation, the most prevalent type of RNA methylation are expected to be used in the treatment of inflammation-based cancers and emerging infectious diseases.

Third, beyond the impact of COVID-19, the changes in people's behavioral patterns globally cannot be ignored [56]. The global COVID-19 pandemic has not only had an imminent impact on routine medical care, including surgery, radiotherapy, and chemotherapy, but has also altered the cancer screening rates, thereby leading to poor early-stage cancer detection [57–59]. The pandemic eventually resulted in a sustained decrease in the number of people referred, diagnosed, and treated for cancer [59]. However, the impact on future cancer morbidity because of a failure of disease detection has been debated, and an analysis of long-term prognosis is awaited. The pandemic had a negative impact on cancer screening in primary care practice, but some were able to mitigate the effects by shifting their focus to processes that do not involve in-person office visits [57]. It is suggested that public health efforts are required to address the huge

cancer screening deficit caused by the COVID-19 pandemic, including the increased use of noninvasive screening modalities [58].

METTL3, an RNA MT, is involved in the methylation of mRNA from OGs, which helps in regulating mRNA splicing, stability, and translation. The process is involved in the modulation of the cytoplasmic antiviral innate immune response receptors, such as *RIG-I*, which stimulates IFN response. In addition, as mentioned in the text, recent studies have shown that MT can methylate viral RNA from SARS-CoV-2, which is involved in the *RIG-I*-IFN pathway. MTi can antagonize the activity of MT, such as METTL3.

Abbreviations

COVID-19: coronavirus disease 2019

IFN: interferon

METTL3: methyltransferase-like 3

miRNAs: microRNAs

MT: methyltransferase

PRRs: pattern recognition receptors

RIG-I: retinoic acid-inducible gene I

SARS-CoV-2: severe acute respiratory syndrome-coronavirus-2

TLRs: toll-like receptors

Declarations

Author contributions

Conflicts of interest

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

References

1. Zhao BS, He C. Fate by RNA methylation: m6A steers stem cell pluripotency. Genome Biol. 2015;16:43.
2. Chao Y, Li HB, Zhou J. Multiple functions of RNA methylation in T cells: a review. Front Immunol. 2021;12:627455.
3. Brocard M, Ruggieri A, Locker N. m6A RNA methylation, a new hallmark in virus-host interactions. J Gen Virol. 2017;98:2207–14.
4. Konno M, Taniguchi M, Ishii H. Significant epitranscriptomes in heterogeneous cancer. Cancer Sci. 2019;110:2318–27.
5. Oerum S, Meynier V, Catala M, Tisné C. A comprehensive review of m6A/m6Am RNA methyltransferase structures. Nucleic Acids Res. 2021;49:7239–55.
6. Niu Y, Zhao X, Wu YS, Li MM, Wang XJ, Yang YG. N^6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinformatics. 2013;11:8–17.
7. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N^6-adenosine methylation. Nat Chem Biol. 2014;10:93–5.
8. Meyer KD, Jaffrey SR. The dynamic epitranscriptome: N^6-methyladenosine and gene expression control. Nat Rev Mol Cell Biol. 2014;15:313–26.
9. Meyer KD, Jaffrey SR. Rethinking m6A readers, writers, and erasers. Annu Rev Cell Dev Biol. 2017;33:319–42.
10. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol. 2011;7:885–7.
11. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell. 2013;49:18–29.
12. Alarcón CR, Goodarzi H, Lee H, Liu X, Tavazoie S, Tavazoie SF. HNRNPA2B1 is a mediator of m6A-dependent nuclear RNA processing events. Cell. 2015;162:1299–308.
13. Fu Y, Zhuang X. m6A-binding YTHDF proteins promote stress granule formation. Nat Chem Biol. 2020;16:955–63.
14. He L, Li H, Wu A, Peng Y, Shu G, Yin G. Functions of N6-methyladenosine and its role in cancer. Mol Cancer. 2019;18:176.
15. Izadpanah A, Rappaport J, Datta PK. Epitranscriptomics of SARS-CoV-2 infection. Front Cell Dev Biol. 2022;10:849298.
16. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021;19:141–54.
17. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011;34:637–50.
18. Zindel J, Kubes P. DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. Annu Rev Pathol. 2020;15:493–518.
19. Liston A, Masters SL. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. Nat Rev Immunol. 2017;17:208–14.

20. Żeromski J, Kaczmarek M, Boruczkowski M, Kierepa A, Kowala-Piaskowska A, Mozer-Lisewska I. Significance and role of pattern recognition receptors in malignancy. Arch Immunol Ther Exp (Warsz). 2019;67:133–41.

21. Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T. TICAM-1, an adaptor molecule that participates in toll-like receptor 3-mediated interferon-β induction. Nat Immunol. 2003;4:161–7.

22. Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, et al. Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-β promoter in the toll-like receptor signaling. J Immunol. 2002;169:6668–72.

23. Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 2005;437:1167–72.

24. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, et al. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol. 2005;6:981–8.

25. Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell. 2005;122:669–82.

26. Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB. VISA is an adapter protein required for virus-triggered IFN-β signaling. Mol Cell. 2005;19:727–40.

27. Liu Y, Olagnier D, Lin R. Host and viral modulation of RIG-I-mediated antiviral immunity. Front Immunol. 2017;7:662.

28. Anchisi, S, Guerra, J, Garcin, D. RIG-I ATPase activity and discrimination of self-RNA *versus* non-self-RNA. mBio. 2015;6:e02349-14.

29. Lu M, Zhang Z, Xue M, Zhao BS, Harder O, Li A, et al. N[6]-methyladenosine modification enables viral RNA to escape recognition by RNA sensor RIG-I. Nature Microbiology. 2020;5:584–98.

30. Li N, Hui H, Bray B, Gonzalez GM, Zeller M, Anderson KG, et al. METTL3 regulates viral m6A RNA modification and host cell innate immune responses during SARS-CoV-2 infection. Cell Rep. 2021;35:109091.

31. Zhang X, Hao H, Ma L, Zhang Y, Hu X, Chen Z, et al. Methyltransferase-like 3 modulates severe acute respiratory syndrome coronavirus-2 RNA N6-methyladenosine modification and replication. mBio. 2021;12:e01067-21.

32. Dixit D, Prager BC, Gimple RC, Poh HX, Wang Y, Wu Q, et al. The RNA m6A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells. Cancer Discov. 2021;11:480–99.

33. Shen C, Sheng Y, Zhu AC, Robinson S, Jiang X, Dong L, et al. RNA demethylase ALKBH5 selectively promotes tumorigenesis and cancer stem cell self-renewal in acute myeloid leukemia. Cell Stem Cell. 2020;27:64–80.e9.

34. Huang H, Weng H, Chen J. m[6]A modification in coding and non-coding RNAs: roles and therapeutic implications in cancer. Cancer Cell. 2020;37:270–88.

35. Dai D, Wang H, Zhu L, Jin H, Wang X. N6-methyladenosine links RNA metabolism to cancer progression. Cell Death Dis. 2018;9:124.

36. Qin Y, Li L, Luo E, Hou J, Yan G, Wang D, et al. Role of m6A RNA methylation in cardiovascular disease (review). Int J Mol Med. 2020;46:1958–72.

37. Zhao F, Xu Y, Gao S, Qin L, Austria Q, Siedlak SL, et al. METTL3-dependent RNA m[6]A dysregulation contributes to neurodegeneration in Alzheimer's disease through aberrant cell cycle events. Mol Neurodegener. 2021;16:70.

38. Yankova E, Blackaby W, Albertella M, Rak J, De Braekeleer E, Tsagkogeorga G, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature. 2021;593:597–601.

39. Li J, Gregory RI. Mining for METTL3 inhibitors to suppress cancer. Nat Struct Mol Biol. 2021;286:460–2.

40. Lee JH, Choi N, Kim S, Jin MS, Shen H, Kim YC. Eltrombopag as an allosteric inhibitor of the METTL3-14 complex affecting the m[6]A methylation of RNA in acute myeloid leukemia cells. Pharmaceuticals (Basel). 2022;15:440.

41. Konno M, Koseki J, Asai A, Yamagata A, Shimamura T, Motooka D, et al. Distinct methylation levels of mature microRNAs in gastrointestinal cancers. Nat Commun. 2019;10:3888.

42. Ohshiro T, Konno M, Asai A, Komoto Y, Yamagata A, Doki Y, et al. Single-molecule RNA sequencing for simultaneous detection of m6A and 5mC. Sci Rep. 2021;11:19304.

43. Zhang L, Sanagapalli S, Stoita A. Challenges in diagnosis of pancreatic cancer. World J Gastroenterol. 2018;24:2047–60.

44. Necula L, Matei L, Dragu D, Neagu AI, Mambet C, Nedeianu S, et al. Recent advances in gastric cancer early diagnosis. World J Gastroenterol. 2019;25:2029–44.

45. Schabath MB, Cote ML. Cancer progress and priorities: lung cancer. Cancer Epidemiol Biomarkers Prev. 2019;28:1563–79.

46. Issa IA, Noureddine M. Colorectal cancer screening: an updated review of the available options. World J Gastroenterol. 2017;23:5086–96.

47. Contreras LM, González-Rivera JC, Baldridge KC, Wang DS, Chuvalo-Abraham J, Ruiz LH. Understanding the functional impact of VOC-ozone mixtures on the chemistry of RNA in epithelial lung cells. Res Rep Health Eff Inst. 2020;201:3–43.

48. Baldridge KC, Zavala J, Surratt J, Sexton KG, Contreras LM. Cellular RNA is chemically modified by exposure to air pollution mixtures. Inhal Toxicol. 2015;27:74–82.

49. Bijland LR, Bomers MK, Smulders YM. Smelling the diagnosis: a review on the use of scent in diagnosing disease. Neth J Med. 2013;71:300–7.

50. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. J Biochem. 2011;150:257–66.

51. Konno M, Asai A, Kitagawa T, Yabumoto M, Ofusa K, Arai T, et al. State-of-the-art technology of model organisms for current human medicine. Diagnostics (Basel). 2020;10:392.

52. Muppidi SS, Katragadda R, Lega J, Alford T, Aidman CB, Moore C. A review of the efficacy of a low-cost cancer screening test using cancer sniffing canines. J Breath Res. 2021;15:024001.

53. Asai A, Konno M, Ozaki M, Kawamoto K, Chijimatsu R, Kondo N, et al. Scent test using *caenorhabditis elegans* to screen for early-stage pancreatic cancer. Oncotarget. 2021;12:1687–96.

54. Moein ST, Hashemian SM, Mansourafshar B, Khorram-Tousi A, Tabarsi P, Doty RL. Smell dysfunction: a biomarker for COVID-19. Int Forum Allergy Rhinol. 2020;10:944–50.

55. Brann DH, Tsukahara T, Weinreb C, Lipovsek M, Van den Berge K, Gong B, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Sci Adv. 2020;6:eabc5801.

56. Ammar A, Brach M, Trabelsi K, Chtourou H, Boukhris O, Masmoudi L, et al. Effects of COVID-19 home confinement on eating behaviour and physical activity: results of the ECLB-COVID19 international online survey. Nutrients. 2020;12:1583.

57. Schad LA, Brady LA, Tumiel-Berhalter LM, Bentham A, Vitale K, Norton A, et al. Impact of COVID-19 on screening rates for colorectal, breast, and cervical cancer: practice feedback from a quality improvement project in primary care. J Patient Cent Res Rev. 2021;8:347–53.

58. Chen RC, Haynes K, Du S, Barron J, Katz AJ. Association of cancer screening deficit in the United States with the COVID-19 pandemic. JAMA Oncol. 2021;7:878–84.

59. Morris EJA, Goldacre R, Spata E, Mafham M, Finan PJ, Shelton J, et al. Impact of the COVID-19 pandemic on the detection and management of colorectal cancer in England: a population-based study. Lancet Gastroenterol Hepatol. 2021;6:199–208.