Urine 5 (2023) 53–56

Contents lists available at ScienceDirect

Urine

journal homepage: www.keaipublishing.com/en/journals/urine/

Commentary: Why have different key biomarkers been reported in the same types of samples from patients with identical diseases?

Takaaki Sato [a,*], Yasuhiko Takahashi [b], Yoichi Mizutani [c]

[a] Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Ikeda, Osaka, 563-8577, Japan
[b] Sumitomo Chemical Co., Ltd., Konohana, Osaka, 554-8558, Japan
[c] Department of Medical Engineering, Faculty of Health Science, Aino University, Ibaraki, Osaka, 567-0012, Japan

ARTICLE INFO

Keywords:
Prostate cancer
Bladder cancer
Urinary volatile biomarkers
Biological information
SPME-GC-MS
Solid-phase microextraction

ABSTRACT

Accurate biomarkers are crucial for early disease detection and improved prognosis. However, the inconsistent reporting of different key biomarkers in the same types of samples from patients with identical diseases in biomarker discovery studies is often questioned. In contrast to such instrumental analyses, the murine olfactory system consistently distinguishes subtle variations in genetically determined individual-unique body odors in urine samples and more pronounced differences in diet-modulated and fluctuating body odors. Interestingly, sniffer mouse behavioral assays revealed that prostate and bladder cancers alter olfactory cues in urine samples to be more intense compared with diet-modulated or genetically determined individual-specific body odors. The causes of inconsistent key biomarkers include high inter-individual and inter-sample variability due to diet-induced metabolites and cosmetic or environmental contaminations. Previously, we proposed experimental procedures tolerant to such noise-like variability or fluctuation, leading to the identification of ten urinary volatile biomarkers for prostate cancer, including 2,6-di(propan-2-yl)phenol as a unique biomarker for bladder cancer. This commentary discusses the theoretical basis of urinary volatile biomarkers and future directions for complementary biomarker development for diagnosis.

1. Data are always correct even in inappropriate experimental designs

Accurate biomarkers are crucial for early disease detection and improved prognosis. However, the inconsistent reporting of different key biomarkers in the same types of samples from patients with identical diseases in biomarker discovery studies is often questioned. Cancer is a complex and dynamic disease and there is a lack of consensus on non-invasively measured volatile biomarkers, even in studies using sniffer dogs. In this commentary, we discuss volatile biomarkers examined in basic studies to distinguish between healthy individuals and those with cancer. For instance, various studies have reported different urinary volatile biomarkers for prostate cancers[1–8] except those from the same laboratory, as well as different volatile biomarkers for lung cancers.[9–12] The inconsistent results regarding key biomarkers can be attributed to inherent heterogeneity and the markedly high inter-individual and inter-sample variability, such as diet-induced metabolites and cosmetic or environmental chemical contaminations. The observed heterogeneity across individual tumors suggests the presence of distinct co-occurring resistance mechanisms within a patient, leading to variations in sensitivity or resistance to specific treatment agents across different regions of the same malignancy at treatment initiation.[13] Consequently, biomarker profiles for identical tissue cancers would differ due to inherent heterogeneity. Notably, humans exhibit greater variability in body odor owing to various diets and genetic backgrounds compared with animals with regular diets and congenic backgrounds. The extent to which each factor alters olfactory cues in urine samples warrants further investigation.

Subtle variations in urinary olfactory cues can be sensitively discriminated by dogs, rats, and mice with over 800 olfactory receptor repertoires. The urinary olfactory cues likely consist of eight elemental components: (1) urine-common odors, (2) genetically determined individual-unique body odors,[14] (3) disease-altered body odors, (4) occult blood odors, (5) diet-induced odors, (6) drug metabolite odors, (7) odors of cosmetic chemical contaminations, and (8) odors of environmental chemical contaminations. Notably, urinary odors exhibit greater dietary variations than genetically determined individual-unique body odors,[15] and diet significantly modifies the odors of exhaled breath.[16] Solid-phase microextraction-gas chromatography-mass spectroscopy

* Corresponding author. National Institute of Advanced Industrial Science and Technology, Ikeda, Osaka, 563-8577, Japan.
E-mail address: taka-sato@aist.go.jp (T. Sato).

https://doi.org/10.1016/j.urine.2023.09.001
Received 7 September 2023; Received in revised form 29 September 2023; Accepted 29 September 2023
Available online 2 October 2023

(SPME-GC-MS) analysis has revealed the presence of environmental chemical contaminations in urinary volatile compounds originating from walls or paints in the urine sampling room.[9] Building upon our previous evaluation of the relative intensities of urinary elemental odors,[6,17] a mouse behavioral assay established the increasing order of 2nd–6th element-related urinary odor as genetically determined individual-unique body odor < dietary odor variation < bladder cancer < occult blood < prostate cancer (after neoadjuvant endocrine therapy) < antibiotic drug metabolites < prostate cancer. Notably, different odorants are often perceived with varying relative odor intensities, even at the same concentration. Furthermore, important biomarkers can sometimes be minor metabolites under healthy or diseased conditions. Thus, scientists must exercise caution when designing experimental procedures to detect crucial biomarkers, aiming to minimize high inter-individual and inter-sample variability. Inadequate variability- or fluctuation-tolerant experimental procedures can lead to different key biomarkers for the same diseases, implying that some may be false even if they appear correct under specific experimental conditions.

To address the second origin of inconsistent key biomarkers, we proposed utilizing a mixture of 25 equal-volume urine samples (5 patients × 5 samples). This approach, similar to data averaging in respective studies, can theoretically improve target-to-background ratios by up to ≤25–5-fold. This helps reduce diet-induced background variability by ≥ 1/25-fold and variability in individual-unique body odor profiles with cosmetic or environmental chemical contaminations by ≥ 1/5-fold against constant target biomarkers in urine samples.[6,17] Interestingly, among our identified ten urinary volatile biomarkers for prostate cancer (Fig. 1), three (dimethyl succinate, acetophenone and phenol) have been reported as biomarkers for lung cancer based on their significant increase in the culture medium of lung carcinoma A549 cells.[10] Additionally, two biomarkers (2-phenyl-2-propanol and 2,6-di(propan-2-yl)phenol, also known as propofol or Diprivan, an intravenous anesthetic) have been reported as urinary volatile biomarkers for lung cancer,[11] whereas one biomarker (2,6-xylidine or 2,6-dimethylaniline) has been reported as a hemoglobin adduct biomarker for bladder cancer.[18,19] Given the low probability of multiple biomarker matches across different types of cancers, the presence of these six biomarkers in two out of three different cancers suggests shared biomarker synthesis mechanisms in tumor cells of various tissue origins.

2. The strategy of the sensory system to overcome information redundancy

The advantage of urine sample mixtures over high inter-individual and inter-sample variability is similar to the strategy employed by the sensory systems. This system allows for the extraction of both slightly different and common information from overlapping receptor signals in a robust manner. For example, in human color vision, the discrimination between a carrot and a mandarin orange is facilitated by the different relative intensities of red and yellow elemental colors. The yellow (Y) elemental color, as the common information, is obtained by summing the signals from L (red, R) and M (green, G) cone photoreceptor cells, which have sensitivity peaks shifted by approximately 30 nm between the L and M cone cells with an overlap of approximately 100 nm in their sensitive wavelength range.[20] By subtracting the signal of the Y elemental color, the L-cone-unique R and M-cone-unique G elemental colors are extracted from the signals of L and M cone cells, respectively.[21] Through the extraction of these three elemental colors (Y, R, and G) using the two redundant L- and M-cone signals, the colors of carrot and mandarin orange can be easily and quantitatively distinguished based on their reddish and yellowish characteristics, respectively. Thus, the four elemental colors, comprising two opponent color pairs (R/G and Y/B), are crucial for humans to accurately discern subtle differences in various colors. Similarly, the urine sample mixture of pre-radical prostatectomy (pre-RP) patients or healthy volunteers serves as a means to extract common biomarkers from urine samples of patients with prostate cancer or healthy volunteers with different genetic, dietary, and environmental backgrounds. By comparing paired olfactory cues or SPME-GC-MS peaks, the subtraction of biomarkers of healthy volunteers reveals in prostate cancer-specific biomarkers. The peak height/area ratios of these biomarkers in patients vs. healthy volunteers would prove to be effective in a manner analogous to the relative intensities of elemental information.

3. Robustness of urinary volatile-mediated biological information

Similar to the extraction of the Y elemental color, the olfactory system extracts elemental odor information by summing signals from cognate olfactory receptors with similar or overlapping tuning. However, there is

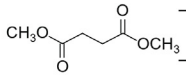
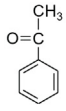
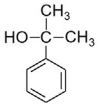
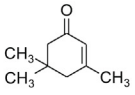
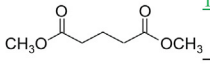
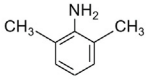
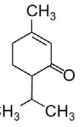
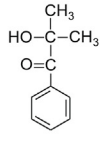
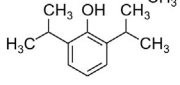
Peak#	Biomarker	#Met	#Isopropyl	Molecular Structure	Fold (pre-RP) mimic	Fold (pre-RP) (post-RP)	Fold (pre-TUR) (post-TUR)
#81	phenol	0	0			38	2.0
					8.5	(1.1)	(0.5)
#101	dimethyl succinate (dimethylated oncometabolite in TCA cycle)	2	0			119	1.8
					35	(3.0)	(2.2)
#104	acetophenone	1	0			62	0.8
					12	(3.0)	(0.9)
#109	2-phyenyl-2-propanol	(2)	0			72	0.5
					7.3	(3.3)	(0.6)
#119	3,5,5-trimethyl-2-cyclohexenone (#81 (Birch reduction) → cyclohexenone)	3	0			2,600	0.6
					1,150	(159)	(1.5)
#123	dimethyl glutarate (dimethylated dehydroxylated oncometabolite)	2	0			76	0.6
					59	(2.2)	(0.7)
#129	2,6-xylidine (dimethylated aniline, causing oxidative DNA damage?)	2	0			161	2.1
					30*(?)	(3.8)	(1.4)
#152	piperitone	1	1			22	0.07
					4.4	(0.1)	(0.08)
#155	2-hydroxy-2-methylpropiophenone	(2)	0			119	1.0
					1.1	(1.1)	(0.5)
#165	2,6-di(propan-2-yl)phenol	0	2			222	40
					1.4	(1.4)	(3.2)

Fig. 1. Molecular structures, numbers of methyl and isopropyl functional groups, and increases (fold) of ten biomarkers in urine samples of pre- and post-resection for prostate (pre- and post-RP) and bladder (pre- and post-TUR) cancers. Most biomarkers are in the methylated or isobutyrated form. >10-fold (red) and 2–10-fold (blue) increases from the peak height of the healthy volunteer are highlighted. *Increase (fold) of #129 estimated by the ratios of the added #101 amounts.[6] RP, radical prostatectomy; TUR, transurethral resection; mimic, a pre-RP urine mixture mimic = post-RP urine mixture + moderate relative amounts of eight biomarkers. (Sato et al., *Intl. J. Cancer Sci. & Therapy* 2021; 3:2–17).

a difference in the weighted elemental information between color vision and olfaction. Unlike simple addition and subtraction in elemental color extraction, the signals of key elemental odors, extracted by summing signals from cognate key olfactory receptors, are significantly enhanced by the feedforward inhibitory system compared to signals from non-key olfactory receptors.[21–25] This signal-enhancing system for key elemental odors enables sniffer mice and dogs to detect prostate cancer-specific olfactory cues of minor but biologically important biomarkers, even at lower concentrations than the more abundant urine-common compounds. As a validation of urinary biomarkers for prostate cancer, sniffer mice successfully discriminated a pre-RP urine mixture mimic (post-RP urine mixture + moderate relative amounts of eight biomarkers) from a post-RP urine mixture (Fig. 1).[6,21] In mice, the extraction of key elemental odors requires summations and subtractions of signals from 29 class-I and 410 class-II key olfactory receptors, whereas auxiliary elemental odors are extracted through signals from 94 class-I and 570 class-II non-key olfactory receptors. This suggests that mice have less than 220 key elemental odors for precise discrimination of olfactory cues. These key elemental odors enable sniffer mice to discern fine differences in the relative intensities of key elemental odors in urinary olfactory cues.

Using an odor discrimination behavior assay with trained sniffer mice, similar to sniffer dogs, it was found that sniffer mice were unable to discriminate subthreshold dietary odor variations between a pair of 10^6-fold diluted urine mixtures.[16] However, in this behavioral assay, the urinary olfactory cue of bladder cancer could be discriminated in 10^6- to 1.3×10^{11}-fold diluted urine mixtures, whereas the urinary olfactory cue of prostate cancer could be discriminated in 10^6- to 1.0×10^{16}-fold diluted urine mixtures containing ppq-level biomarkers in accordance with the Fechner's law that governs semilogarithmic decreases in correct odor choice rates.[6,21] This result highlights the super-sensitivity of sniffer mice to urinary olfactory cues of prostate and bladder cancers, as well as the robustness of urinary volatile-mediated biological information. Furthermore, sniffer mice successfully discriminated the olfactory cues of antibiotic drug metabolites and occult blood in 10^6- to 3.1×10^{15}-fold and 10^6- to 1.0×10^{12}-fold diluted urine mixtures, respectively.[17] The robustness of urinary olfactory cues may explain why dogs relay on urinary odors, rather than sweat odors, to detect traces of territory-invaded competitors. Urine mixtures serve as more stable and reliable samples for non-invasive and disease-discriminative diagnostic tests compared to single urine samples or easily contaminated breath or sweat samples.

4. .Urinary volatile biomarkers for cancers

As mentioned earlier, the six biomarkers for prostate cancer (acetophenone, piperitone, dimethyl succinate, dimethyl glutarate, 2,6-xylidine, 3,5,5-trimethyl-2-cyclohexenone) are also biomarkers for bladder or lung cancer, indicating shared synthetic mechanisms among these biomarkers in different tumor cells. Among the biomarkers for prostate cancers,[6] six biomarkers contain one to three methyl groups, whereas two (piperitone and 2,6-di(propan-2-yl)phenol) have one or two isopropyl groups (Fig. 1). The prevalence of methylated biomarkers suggests abnormal activation of methionine synthases and/or methyltransferases, as well as significantly reduced activity of demethylases.

Cancer cells undergo a metabolic shift from energetic to anabolic metabolism to support uncontrolled cell proliferation.[26] This shift is initiated by oncometabolites, namely succinate or fumarate, which accumulate in the tricarboxylic cycle within the mitochondrion matrix.[27–33] Mutations in isocitrate dehydrogenase (IDH1 and IDH2), on the other hand, lead to the accumulation of another oncometabolite called 2-hydroxyglutarate, through the generation of oxalosuccinate and NADH and subsequent conversion to α-ketoglutarate.[34] The biomarkers dimethyl succinate and dimethyl glutarate may be associated with the accumulation of oncometabolites succinate and 2-hydroxyglutarate, as well as their methylation under increased methylation pressure in tumor cells. Considering that there are shared biomarkers between prostate and bladder cancers, albeit with different profiles of relative increases (fold as shown in Fig. 1), differences in biomarker profiles would be helpful identifying the types of cancers, as opposed to relying solely on simple cut-off values.[6,21]

Interestingly, the biomarker propofol (2,6-di(propan-2-yl)phenol) was detected at the lowest concentration in urine samples of healthy volunteers, approximately 0.01 ppb. This concentration was 1000-fold and 100-fold lower than those of phenol and other seven biomarkers, respectively.[6,21] In the urine mixture of patients with prostate and bladder cancers, propofol increased to 3.3 ppb and 0.6 ppb, respectively.[6,21] It has been reported that propofol upregulates tissue-characteristic miRNAs or other regulating factors, resulting in reduced tumor cell variabilities and invasions in various types of cancers.[35–40] Although the actual concentration of propofol in tumor cells is much higher than those of the urine mixtures, it remains unclear to what extent the biomarker propofol may function as an inhibitor of tumor cell variabilities and invasions.

5. Future study for multiple complementary tests for the diagnosis of early-stage cancers

Prostate-specific antigen (PSA) levels in blood serve as the most widely used biomarkers for prostate cancer. However, elevated PSA levels can also be caused by prostatitis or benign prostatic hyperplasia, leading to that PSA is a sensitivity–specificity trade-off and overdiagnosis of prostate cancer. To improve the diagnostic accuracy for prostate and other cancers, a combination of complementary biomarkers is recommended.[41] Panels of volatile urinary biomarkers have shown enhanced sensitivity (76%–89%) and specificity (83%–90%).[4,5,8] In addition to these volatile urinary biomarkers, non-volatile biomarkers have been proposed as complementary indicators for prostate cancer. These include circulating tumor DNA associated with mutations in tumor suppressor p53 (TP53),[42] androgen receptor,[42,43] ataxia telangiectasia-mutated kinases,[42] the transcription factor MYC,[42] or the E3 ubiquitin ligase adaptor speckle-type POZ protein.[42] Other biomarkers include the prostate cancer antigen 3 (PCA3) score,[43,44] Prostate Health Index (PHI),[43,44] 4 K score,[44] *TMPRSS2:ERG* fusion gene,[43] *PTEN* gene,[43] SelectMDx (transcription factor homeobox C6 [HOXC6] and distal-less homeobox 1 [Dlx1] mRNA levels),[44] ExoDx Prostate Intelliscore (exosomal mRNA of PCA3 and ERG [Vetserythroblastosis virus E26 oncogene homologs] normalized with a control gene, SAM-pointed domain-containing Ets-like factor [SPDEF]),[44] exosomal noncoding RNAs (10 miRNAs in plasma or serum, 19 miRNAs in urine, 3 long-noncoding RNAs in plasma or urine),[45] and serum mRNA of caspase-8 (CASP-8).[46]

Urine samples exhibit stability dependent on physical conditions and serve as a source of highly sensitive and non-invasive biomarkers for diagnosing a wide range of diseases. Further information can be found in a recent review article.[47] In the future, we anticipate the development of urinary biomarker complementary panel tests for early-stage diagnosis of various diseases.

CRediT authorship contribution statement

Takaaki Sato: Formal analysis, Investigation, Project administration, Writing – review & editing, Methodology. **Yasuhiko Takahashi:** Formal analysis, Investigation, Project administration, Writing – review & editing, Methodology. **Yoichi Mizutani:** Formal analysis, Investigation, Project administration, Writing – review & editing, Methodology.

Declaration of competing interest

Our organizations have filed patent applications (the authors are listed as inventors) of the biomarkers, which may be not affected by publication. The authors declare no other conflicts of interest.

References

1. Bax C, Taverna G, Eusebio L, Sironi S, Grizzi F, et al. Innovative diagnostic methods for early prostate cancer detection through urine analysis: a Review. *Cancers*. 2018; 10:123. https://doi.org/10.3390/cancers10040123.
2. Porto-Figueira P, Pereira JAM, Câmara JS. Exploring the potential of needle trap microextraction combined with chromatographic and statistical data to discriminate different types of cancer based on urinary volatomic biosignature. *Anal Chim Acta*. 2018;1023:53–63. https://doi.org/10.1016/j.aca.2018.04.027.
3. Jiménez-Pacheco A, Salinero-Bachiller M, Iribar MC, López-Luque A, Miján-Ortiz JL, et al. Furan and p-xylene as candidate biomarkers for prostate cancer. *Urol Oncol*. 2018;36. https://doi.org/10.1016/j.urolonc.2017.12.026, 243.e21-243.e27.
4. Lima AR, Pinto J, Azevedo AI, Barros-Silva D, Jerónimo C, et al. Identification of a biomarker panel for improvement of prostate cancer diagnosis by volatile metabolic profiling of urine. *Br J Cancer*. 2019;121:857–868. https://doi.org/10.1038/s41416-019-0585-4.
5. Lima AR, Pinto J, Carvalho-Maia C, Jerónimo C, Henrique R, et al. A Panel of urinary volatile biomarkers for differential diagnosis of prostate cancer from other urological cancers. *Cancers*. 2020;12:2017. https://doi.org/10.3390/cancers12082017.
6. Sato T, Nonomura M, Yoneda K, Mizutani S, Mizutani Y. Prostate cancer-induced changes in urinary odors at biomarker concentrations of ppq with validation by sniffer mouse behavioural assays. *Int J Cancer Sci Ther*. 2021;3:2–17. https://doi.org/10.31487/j.IJCST.2021.01.02.
7. Liu Q, Fan Y, Zeng S, Zhao Y, Yu L, et al. Volatile organic compounds for early detection of prostate cancer from urine. *Heliyon*. 2023;9:e16686. https://doi.org/10.1016/j.heliyon.2023.e16686.
8. Woollam M, Siegel AP, Munshi A, Liu S, Tholpady S, et al. Canine-inspired chemometric analysis of volatile organic compounds in urine headspace to distinguish prostate cancer in mice and men. *Cancers*. 2023;15:1352. https://doi.org/10.3390/cancers15041352.
9. Matsumura K, Opiekun M, Oka H, Vachani A, Albelda SM, et al. Urinary volatile compounds as biomarkers for lung cancer: a proof of principle study using odor signatures in mouse models of lung cancer. *PLoS One*. 2010;5:e8819. https://doi.org/10.1371/journal.pone.0008819.
10. Hanai Y, Shimono K, Oka H, Baba Y, Yamazaki K, et al. Analysis of volatile organic compounds released from human lung cancer cells and from the urine of tumor-bearing mice. *Cancer Cell Int*. 2012;12:7. https://doi.org/10.1186/1475-2867-12-7.
11. Hanai Y, Shimono K, Matsumura K, Vachani A, Albelda S, et al. Urinary volatile compounds as biomarkers for lung cancer. *Biosci Biotechnol Biochem*. 2012;76: 679–684. https://doi.org/10.1271/bbb.110760.
12. Janssens E, van Meerbeeck JP, Lamote K. Volatile organic compounds in human matrices as lung cancer biomarkers: a systematic review. *Crit Rev Oncol Hematol*. 2020;153:103037. https://doi.org/10.1016/j.critrevonc.2020.103037.
13. Holbeck SL, Camalier R, Crowell JA, Govindharajulu JP, Hollingshead M, et al. The National Cancer Institute ALMANAC: a comprehensive screening resource for the detection of anticancer drug pairs with enhanced therapeutic activity. *Cancer Res*. 2017;77:3564–3576. https://doi.org/10.1158/0008-5472.CAN-17-0489.
14. Schaefer ML, Yamazaki K, Osada K, Restrepo D, Beauchamp GK. Olfactory fingerprints for major histocompatibility complex-determined body odors II: relationship among odor maps, genetics, odor composition, and behavior. *J Neurosci*. 2002;22:9513–9521. https://doi.org/10.1523/JNEUROSCI.22-21-09513.2002.
15. Kwak J, Willse A, Matsumura K, Opiekun MC, Yi W, et al. Genetically-based olfactory signatures persist despite dietary variation. *PLoS One*. 2008;3:e3591. https://doi.org/10.1371/journal.pone.0003591.
16. Fischer S, Bergmann A, Steffens M, Trefz P, Ziller M, et al. Impact of food intake on in vivo VOC concentrations in exhaled breath assessed in a caprine animal model. *J Breath Res*. 2015;9:047113. https://doi.org/10.1088/1752-7155/9/4/047113.
17. Sato T, Katsuoka Y, Yoneda K, Nonomura M, Uchimoto S, et al. Sniffer mice discriminate urine odours of patients with bladder cancer: a proof-of-principle study for non-invasive diagnosis of cancer-induced odours. *Sci Rep*. 2017;7:14628. https://doi.org/10.1038/s41598-017-15355-z.
18. Gan J, Skipper PL, Gago-Dominguez M, Arakawa K, Ross RK, et al. Alkylaniline-hemoglobin adducts and risk of non-smoking related bladder cancer. *J Natl Cancer Inst*. 2004;96:1425–1431. https://doi.org/10.1093/jnci/djh274.
19. Tao L, Day BW, Hu B, Xiang YB, Wang R, et al. Elevated 4-aminobiphenyl and 2,6-dimethylaniline hemoglobin adducts and increased risk of bladder cancer among lifelong nonsmokers—the Shanghai Bladder Cancer Study. *Cancer Epidemiol Biomarkers Prev*. 2013;22:937–945. https://doi.org/10.1158/1055-9965.EPI-12-1447.
20. Sharpe LT, Stockman A, Jägle H, Nathans J. Opsin genes, cone photopigments, color vision, and color blindness. In: Gegenfurtner KR, Sharpe LT, eds. *Color Vision from Genes to Perception*. Cambridge, UK: Cambridge University Press; 1999:3–52.
21. Sato T, Matsukawa M, Iijima T, Mizutani Y. Hierarchical elemental odor coding for fine discrimination between enantiomer odors or cancer-characteristic odors. *Front Behav Neurosci*. 2022;16:849864. https://doi.org/10.3389/fnbeh.2022.849864.
22. Sato T, Kobayakawa R, Kobayakawa K, Emura M, Itohara S, et al. Supersensitive detection and discrimination of enantiomers by dorsal olfactory receptors: evidence for hierarchical odour coding. *Sci Rep*. 2015;5:14073. https://doi.org/10.1038/srep14073.
23. Sato T, Kawasaki T, Mine S, Matsumura H. Functional role of the C-terminal amphipathic helix 8 of olfactory receptors and other G protein-coupled receptors. *Int J Mol Sci*. 2016;17:1930. https://doi.org/10.3390/ijms17111930.
24. Sato T. Conserved 2nd residue of helix 8 of GPCR may confer the subclass-characteristic and distinct roles through a rapid initial interaction with specific G proteins. *Int J Mol Sci*. 2019;20:1752. https://doi.org/10.3390/ijms20071752.
25. Sato T, Kajiwara R, Takashima I, Iijima T. A novel method for quantifying similarities between oscillatory neural responses in wavelet time-frequency power profiles. *Brain Res*. 2016;1636:107–117. https://doi.org/10.1016/j.brainres.2016.01.054.
26. Vazquez A, Kamphorst JJ, Markert EK, Schug ZT, Tardito S, Gottlieb E. Cancer metabolism at a glance. *J Cell Sci*. 2016;129:3367–3373. https://doi.org/10.1242/jcs.181016.
27. Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, et al. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell*. 2005;8:143–153. https://doi.org/10.1016/j.ccr.2005.06.017.
28. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell*. 2005;7:77–85. https://doi.org/10.1016/j.ccr.2004.11.022.
29. Kastrati I, Siklos MI, Calderon-Gierszal EL, El-Shennawy L, Georgieva G, et al. Dimethyl fumarate inhibits the nuclear factor κB pathway in breast cancer cells by covalent modification of p65 protein. *J Biol Chem*. 2016;291:3639–3647. https://doi.org/10.1074/jbc.M115.679704.
30. Eijkelenkamp K, Osinga TE, Links TP, van der Horst Schrivers ANA. Clinical implications of the oncometabolite succinate in SDHx-mutation carriers. *Clin Genet*. 2020;97:39–53. https://doi.org/10.1111/cge.13553.
31. Dalla-Pozza E, Dando I, Pacchiana R, Liboi E, Scupoli MT, et al. Regulation of succinate dehydrogenase and role of succinate in cancer. *Semin Cell Dev Biol*. 2020; 98:4–14. https://doi.org/10.1016/j.semcdb.2019.04.013.
32. Mu X, Zhao T, Xu C, Shi W, Geng B, et al. Oncometabolite succinate promotes angiogenesis by upregulating VEGF expression through GPR91-mediated STAT3 and ERK activation. *Oncotarget*. 2017;8:13174–13185. https://doi.org/10.18632/oncotarget.14485.
33. Nowicki S, Gottlieb E. Oncometabolites: tailoring our genes. *FEBS J*. 2015;282: 2796–2805. https://doi.org/10.1111/febs.13295.
34. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462:739–752. https://doi.org/10.1038/nature08617.
35. Gong T, Ning X, Deng Z, Liu M, Zhou B, et al. Propofol-induced miR-219-5p inhibits growth and invasion of hepatocellular carcinoma through suppression of GPC3-mediated Wnt/β-catenin signalling activation. *J Cell Biochem*. 2019;120: 16934–16945. https://doi.org/10.1002/jcb.28952.
36. Du Y, Zhang X, Zhang H, Chen Y, Zhu S, et al. Propofol modulates the proliferation, invasion and migration of bladder cancer cells through the miR-145-5p/TOP2A axis. *Mol Med Rep*. 2021;23:439. https://doi.org/10.3892/mmr.2021.12078.
37. Yang C, Gao J, Yan N, Wu B, Ren Y, et al. Propofol inhibits the growth and survival of gastric cancer cells in vitro through the upregulation of ING3. *Oncol Rep*. 2017;37: 587–593. https://doi.org/10.3892/or.2016.5218.
38. Yang N, Liang Y, Yang P, Yang T, Jiang L. Propofol inhibits lung cancer cell viability and induces cell apoptosis by upregulating microRNA-486 expression. *Braz J Med Biol Res*. 2017;50:e5794. https://doi.org/10.1590/1414-431x20165794.
39. Yu B, Gao W, Zhou H, Miao X, Chang Y, et al. Propofol induces apoptosis of breast cancer cells by downregulation of miR-24 signal pathway. *Cancer Biomarkers*. 2018; 21:513–519. https://doi.org/10.3233/cbm-170234.
40. Lu H, Zheng G, Gao X, Chen C, Zhou M, Zhang L. Propofol suppresses cell viability, cell cycle progression and motility and induces cell apoptosis of ovarian cancer cells through suppressing MEK/ERK signaling via targeting circVPS13C/miR-145 axis. *J Ovarian Res*. 2021;14:30. https://doi.org/10.1186/s13048-021-00775-3.
41. Khoo A, Liu LY, Nyalwidhe JO, Semmes OJ, Vesprini D, et al. Proteomic discovery of non-invasive biomarkers of localized prostate cancer using mass spectrometry. *Nat Rev Urol*. 2021;18:707–724. https://doi.org/10.1038/s41585-021-00500-1.
42. Chen G, Jia G, Chao F, Xie F, Zhang Y, et al. Urine- and blood-based molecular profiling of human prostate cancer. *Front Oncol*. 2022;12:759791. https://doi.org/10.3389/fonc.2022.759791.
43. Filella X, Fernández-Galan E, Fernández-Bonifacio R, Foj L. Emerging biomarkers in the diagnosis of prostate cancer. *Pharmgenomics Pers Med*. 2018;11:83–94. https://doi.org/10.2147/PGPM.S136026.
44. Farha MW, Salami SS. Biomarkers for prostate cancer detection and risk stratification. *Ther Adv Urol*. 2022;14:17562872221103988. https://doi.org/10.1177/17562872221103988.
45. Movahedpour A, Khatami SH, Karami N, Vakili O, Naeli P, et al. Exosomal noncoding RNAs in prostate cancer. *Clin Chim Acta*. 2022;537:127–132. https://doi.org/10.1016/j.cca.2022.10.018.
46. Liu S, Garcia-Marques F, Zhang CA, Lee JJ, Nolley R, et al. Discovery of CASP8 as a potential biomarker for high-risk prostate cancer through a high-multiplex immunoassay. *Sci Rep*. 2021;11:7612. https://doi.org/10.1038/s41598-021-87155-5.
47. Balhara N, Devi M, Balda A, Phour M, Giri A. Urine; a new promising biological fluid to act as a non-invasive biomarker for different human diseases. *Urine*. 2023;5:40–52. https://doi.org/10.1016/j.urine.2023.06.001.