

REVIEW

# Digging deeper into volatile organic compounds associated with cancer

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## Abstract

Volatile organic compounds (VOCs), produced and emitted through the metabolism of cancer cells or the body's immune system, are considered novel cancer biomarkers for diagnostic purposes. Of late, a large number of work has been done to find a relationship between VOCs' signature of body and cancer. Cancer-related VOCs can be used to detect several types of cancers at the earlier stages which in turn provide a significantly higher chance of survival. Here we aim to provide an updated picture of cancer-related VOCs based on recent findings in this field focusing on cancer odor database.

**Keywords:** cancer odor; cancer diagnosis; volatile organic compounds; lung cancer; aldehydes

## Introduction

Cancer is one of the leading causes of death and a major public health problem worldwide. The development of a reliable method for diagnosis and treatment of cancer has been the subject of numerous recent studies [1–3].

A solid body of research literature shows that the chance of survival in people with different types of cancer is increased with earlier detection. Today, most diagnostic tools are unable to detect cancers at the very early stages of disease progression. Moreover, some of those tools are invasive and may present clinical risks for the patient. Therefore, the demand for alternative methods for cancer diagnosis has increased in recent years [4–7].

The discovery of cancer biomarkers opened a new realm of possibilities for cancer detection. Various types of biomarkers including proteins, peptides, metabolites, DNA, RNA, and whole cells are thought to assist in the diagnosis of cancer [8, 9]. With the development of technology and with a greater understanding of cancer itself, the discovery of biomarkers has accelerated tremendously. As a result, a number of potential biomarkers are being reported every year and some of them have been introduced into clinical practice. Finding potential biomarkers that are able to be used for the detection of cancers at the onset of disease holds promise for the future treatment of cancers [9].

To achieve this goal, different methods have been applied up to now. The detection of different types of cancer, including lung [10, 11], colon [12, 13], breast [14, 15], pancreatic [16, 17],

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prostate [12, 18], and head and neck [19], using volatile organic compounds (VOCs), has attracted the attention of scientists in recent years [15, 17, 20–22]. These studies usually involve profiling VOCs present in biological samples from cancer patients and healthy people and comparing the pattern of VOCs in healthy and cancer groups.

The entire set of VOCs generated by an organism is called “volatilome” or “volatome” and the study of volatilome is known as “volatilomics” [23]. VOCs generated through the metabolism of cells release into the blood and are excreted through the exhaled breath or body fluids. The volatilomic profile of different biological matrices can be efficiently identified by analytical methods.

It has been well documented that VOCs can give useful information about the metabolic state of an organism [24, 25]. The VOCs of our body reflect biochemical reactions caused by biological activities such as cell death, oxidative stress, or inflammation. Disease-related VOCs may be part of the cascade of the reactions that occur during the response of the body to the damage [24]. Therefore, the VOCs can be used as novel biomarkers for diagnostic purposes.

In the last decade, a considerable amount of effort has been directed toward finding the relationship between the VOCs’ signature of the body and the presence of cancer. There are many reports investigating VOCs associated with different types of cancers, known as cancer odor; however, these works only refer to their results or compare their results with other limited works. There are no comprehensive analyses of all of the available data about cancer-related VOCs. To address this problem, we have developed cancer odor database (COD), a comprehensive database of cancer-related VOCs [26].

The COD is a web-based database that contains comprehensive information of the VOCs associated with cancer manually extracted from literature. The database contains >1300 records with 19 critical features for each record and provides an excellent overview of volatile organic metabolites of cancer. The COD is freely available for noncommercial purposes online at <http://bioinf.modares.ac.ir/software/cod>. In this article, we will focus on VOCs associated with different types of cancer obtained from the COD database and review their origins, biological matrices, and methods of detection.

## Biological matrices

VOCs produced by the body are first released into the circulatory system. They can then enter the air in the lungs or biofluids. Research on cancer-related VOCs has been performed using various human matrices including blood, breath, urine, bile, feces, saliva, and vocal fold lesions.

Evaluation of the COD database indicates that the most extensive research in cancer-related VOCs has been on exhaled breath, and especially for the detection of lung cancer (Fig. 1). Noninvasive methods, like breath analysis, are preferred for diagnosis due to the ease of sampling procedures, and low cost, the breath can be sampled and analyzed in real time [27]. Finally, the breath is less complex than other matrices like blood, which eliminates the need for preprocessing of samples.

Blood has been used as metrics for VOC collection in a number of cancer studies [28–30]. Obtaining blood samples can be costly and time-consuming and may not be well tolerated by patients in comparison to obtaining breath or urine samples. It is worth noting that changes in the temperature and pH of blood samples can alter the VOC profile.

Several studies have also investigated VOCs in urine or feces samples of patients with various types of cancers [31, 32]. These two matrices are available in large volumes and their sample collection is noninvasive.

The kidneys concentrate analytes in the urine before it is excreted from the body which is an advantage over other biofluids. However, the drugs administered to a patient might influence VOCs in the urine. There are also some problems that may limit using feces as matrices, e.g. VOCs in the feces may be affected or generated by intestinal flora or infectious diseases.

There are several studies investigating cancer-related VOCs *in vitro*. *In vitro* experiments allow for better control of experimental variables. In addition, the results of *in vitro* studies can be easily interpreted, due to the absence of various parameters such as gender and age. Investigation of tumor cells *in vitro* makes it easier to directly recognize VOCs related to cancer among a large number of metabolites produced by the cells [33, 34]. On the contrary, several VOCs are produced through reactions in other organs. Therefore, studying the cancer cells alone will not capture information arising from the secondary interaction of VOCs with other organs [35–37].

The concentration of VOCs in human breath, blood, and urine is in the range of ppm to ppt [38]. Therefore, a preconcentration step is usually necessary prior to the analysis. In recent years, considerable efforts have been directed toward improvements in the sampling of volatile compounds and preconcentration technologies. Solid-phase microextraction (SPME) is a simple, fast, economic, and solvent-free preconcentration technique, which is widely used for the analysis of VOCs in biological samples. SPME is typically coupled with a separation technique for analysis of biological samples.

## Analytical methods

Techniques for detection of cancer-related VOCs in biological samples can be broadly divided into two groups: those using analytical instruments and those using sensor and electronic nose systems [39].

Various analytical instruments for determining VOCs have been used including gas chromatography–mass spectrometry, ion mobility spectrometry, field asymmetric ion mobility spectrometry, selected ion flow tube mass spectrometry (SIFT-MS), proton transfer reaction–mass spectrometry (PTR-MS), gas chromatography–flame ionization detection, and comprehensive 2D gas chromatography [7].

Based on information from the COD database, gas chromatography coupled to mass spectrometry has been the main analytical method for detection of VOCs, however, other methods like PTR-MS and SIFT-MS have also been widely employed [26].

The abovementioned methods are commonly combined with separation and preconcentration methods like solid-phase extraction [40, 41]. Two points should be taken into account with regard to preconcentration methods. First, the presence of exogenous compounds must be minimized through experimental procedures. And second, the pH of biological fluids is another parameter that must be considered because it may influence the microextraction process [42–44].

Recently, sensors and electronic noses have shown promise as an alternative to traditional diagnostic tools. They have attracted a great deal of interest due to the advantages of high sensitivity, portability, low cost, and ease-of-use [45, 46]. Sensor-based techniques have great potential in clinical point-of-care use [47]. Various types of gas sensors including metal oxide chemiresistive sensors, nanomaterial-based chemiresistive

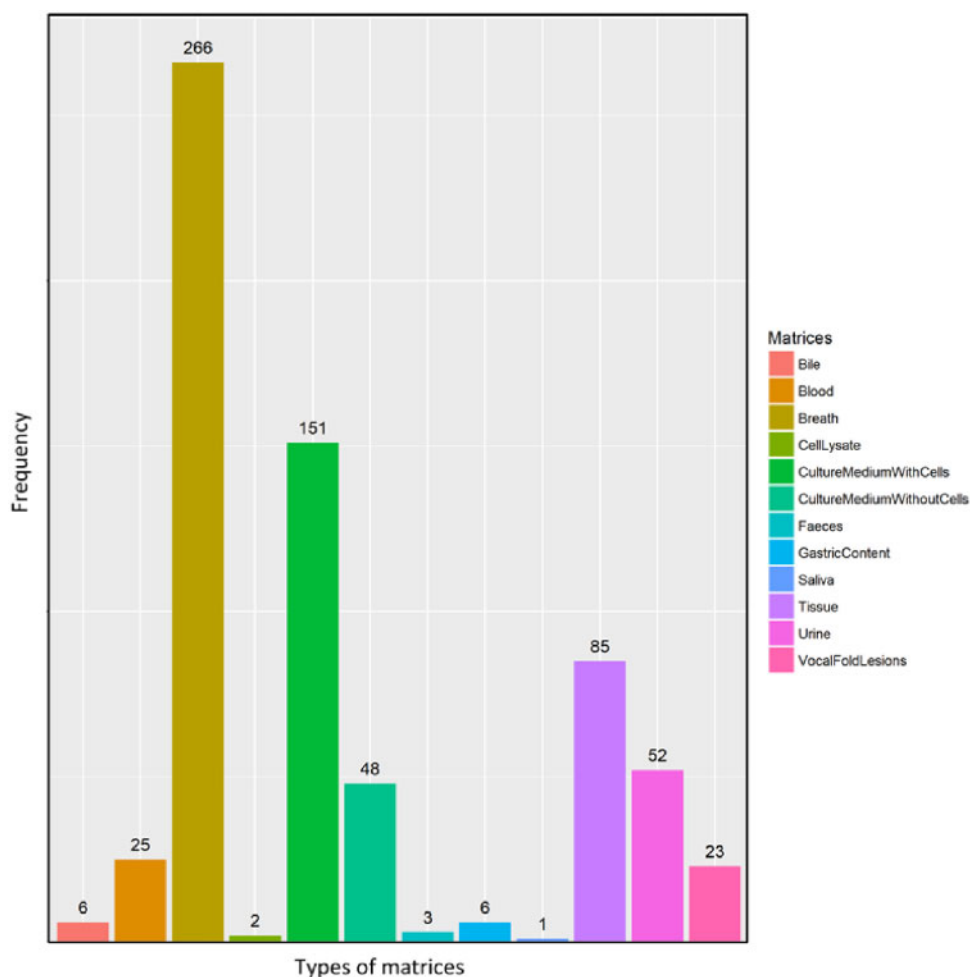


Figure 1: Frequency of VOCs detected in various types of biological matrices.

sensors, piezoelectric sensors, colorimetric sensors, metal-organic frameworks, silicon nanowire field-effect transistor, and olfactory receptor-based sensors have been developed in order to detect cancer-related VOCs [7].

### Important cancer-related VOCs and their origin

The primary goal of this study was to find potentially significant cancer-related VOCs based on existing reports. Cancer biomarkers can be classified into two main categories: general cancer biomarkers and biomarkers for a specific cancer type. An analysis of the COD data shows that some VOCs only contribute to a particular type of cancer and can be considered a specific biomarker for that type of cancer, while other VOCs are associated with several types of cancers and can be considered as generic cancer biomarkers.

In order to find these cancer-related VOCs, the network of VOCs and their corresponding cancer type was constructed. Then, VOCs that contribute to more than three types of cancer were analyzed. Figure 2 shows the bipartite VOCs-cancer network corresponding to the VOCs observed in more than two different types of cancer. The blue circles and orange diamond represent VOCs and cancer types, respectively, and edges correspond to the association of VOCs with cancers.

Figure 3 shows the VOCs ranked based on their association with three to eight types of cancer. It was observed that the

VOCs in Fig. 3 can be classified by their chemical functional into five main groups: aldehydes (heptanal, hexanal, decanal, nonanal, pentanal, and octanal), ketones (acetone, 3-heptanone, 2-butanone, and cyclohexanone), alcohols (2-ethylhexanol), hydrocarbons (dodecane, 3-methylehexan, 4-methyloctane, and 2,2-dimethyldecane), and aromatic compounds (1,2,4-trimethylbenzene, 1-methyl-4-propan-2-ylbenzene, and *p*-xylene).

Indeed, these VOCs may be involved in some pathways involved in various types of cancer and can be considered as excellent general cancer biomarkers for early detection or risk prediction. A combination of general cancer biomarkers and specific biomarkers would be very promising for cancer detection.

The origin of many of the VOCs observed in different cancers has not been clearly defined. Understanding the metabolic pathways that lead to the production or elimination of these VOCs will result in a better understanding of the biochemical changes that occur in cancers. In the next section, we aim to shed light on the abovementioned significant VOCs and elucidate the biochemical pathways that they are involved in.

### Aldehydes

As shown in Fig. 3, all the VOCs associated with more than five types of cancers (heptanal, hexanal, decanal, and nonanal)

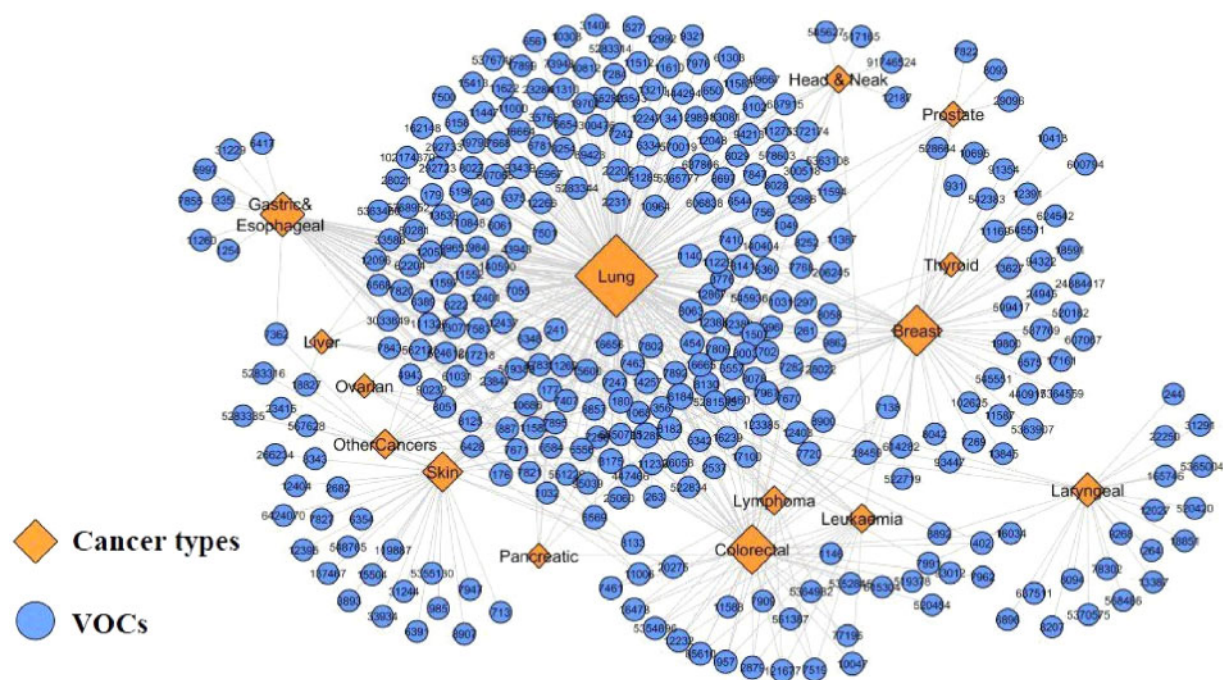


Figure 2: The network representations of cancer-related VOCs (shown by their PubChem ID) and cancer types obtained from analyzing the COD database.

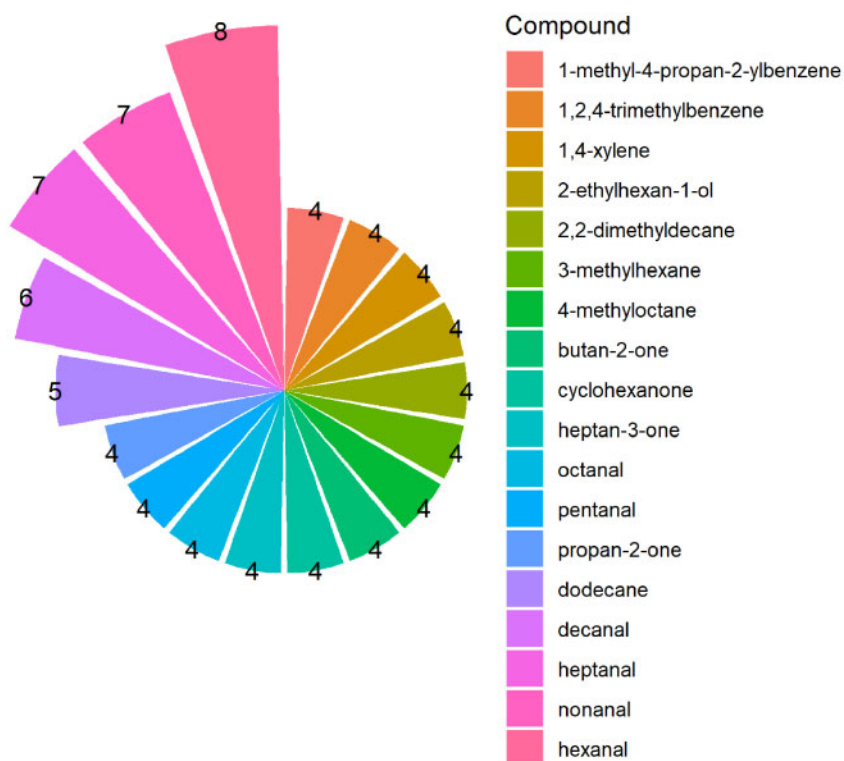


Figure 3: VOCs related to four or more than four types of cancer. The number of different types of cancer in which the VOCs are contributed is indicated.

belong to the chemical class of aldehydes. Among these cancer-related aldehydes, hexanal has been reported for eight different cancer types thus showing its importance as a potential cancer biomarker. Several aldehydes including acetaldehyde, benzaldehyde, hexanal, heptanal, and octanal can be found in all

human body matrices including breath, blood, saliva, skin secretions, urine, and feces [38].

A number of studies suggest a significant enhancement of aldehydes in cancer patients compared to healthy controls [48, 49]. Aldehydes are slightly soluble in blood and can be found in



breath in just minutes after their formation in tissues [50]. There are some reports implying that there is no relationship between the level of aldehydes (in breath) and the age or gender of the patient [51–54].

Aldehydes are produced by different mechanisms. They can originate from dietary sources [55], metabolized alcohols, and smoking. One of the main sources of aldehydes is their generation as secondary oxidation products. Production of monofunctional C3–C10 aldehydes such as *n*-hexanal, *n*-heptanal, *n*-nonanal, and *n*-decanal is related to the reduction of hydroperoxides by cytochrome P450 (CYP450) through the lipid-oxidation of omega-3 and -6 polyunsaturated fatty acids (PUFAs) like linoleic or arachidonic acid [56–58].

CYP450 enzymes, a multigene family of constitutive and inducible enzymes found in many tissues with the highest concentration and activity in the liver, play a central role in human physiology. CYP is responsible for the oxidative metabolism of various endogenous and exogenous compounds. CYP450 enzymes play an important role in the Phase I metabolism of a broad range of different compounds. They oxidize these compounds to more hydrophilic products and hence facilitate their excretion [59, 60].

The P450s play a key role in cancer formation and are involved in tumor initiation or promotion, because of their ability to activate or deactivate most carcinogens. Reactive oxygen species (ROS) can be generated from the reactions mediated by CYP450s which are known to be overexpressed in cancer cells. It has been shown that the CYP450 family 1B1 is overexpressed in several types of tumor cells and associated with angiogenesis. The CYP2E1, another CYP450 family member, is one of the most active members of the family in terms of ROS production. They induce the generation of ROS which causes effects such as DNA damage, autophagy, unfolded protein response, enhanced angiogenic responses, and endoplasmic reticulum (ER) stress. CYP2E1 gene overexpression has been observed in malignant tissues in comparison with normal ones resulting in an increased level of inflammatory cytokines in the tumor microenvironment [61]. CYP2E1-mediated ROS generation can contribute to tumor development through different pathways [60]. Targeting of CYP450s in cancer therapy is very attractive.

It has also been found that the amount of saturated lipids in cancer cell membranes is greater than that in normal cells [62, 63]. Therefore, increased aldehyde production in cancer patients may be due to the changes in membrane lipid composition as well as increased oxidative stress in tumor cells. Furthermore, increased levels of certain unsaturated fatty acids in the membranes of tumor cells may increase the production of certain aldehydes through lipid peroxidation.

Aldehyde dehydrogenases (ALDHs) and alcohol dehydrogenases (ADHs) are two abundant enzymes in the human liver [64, 65]. Aldehydes can be irreversibly oxidized to carboxylic acids by ALDHs or reduced to their corresponding alcohols by ALHs [64].

ADH, which catalyzes the conversion of alcohol to aldehyde, is thought to play a role in the formation of both local and distant metastasis [66]. It has been found that ADH in the ovary of rats can metabolize alcohols to toxic aldehydes which could lead to cell damage [67–69]. Comparison of aldehydes levels in the reported cancer cell lines and cell lines treated with ADH inhibitors like 4-methylpyrazole might aid in a detailed understanding of the role of ADH. It has been demonstrated that downregulation of 10-formyltetrahydrofolate dehydrogenase in ovarian cancer cells leads to increased aldehydes levels [70]. Overexpression of ALDHs has also been shown in lung cancer

cells [71]. Therefore, the concentration of cancer-related aldehydes can be reduced or increased in different reported cases.

Due to a decreased level of some types of aldehydes in the headspace of cancer cells compared to medium control, it has been suggested that these aldehydes are consumed or taken up by the cancer cells. Uptake of several volatile compounds has been reported in various cancer cell lines such as A-549, RPE, BEAS2B, CALU-1, and NCI-H1666 [72–75].

Saturated aldehydes are known to be taken up more easily than unsaturated ones. Hexanal can be metabolized in HepG2 cell cultures [76]. The uptake of volatiles by HepG2 cells from the culture medium may provide insight into the metabolism of these cells. Aldehydes can be metabolized by human hepatocellular carcinoma cells [76, 77]. However, the consumption of aldehydes in the headspace of the cells *in vitro* is not specific for cancer cells and can be seen in noncancerous cells [73, 74, 78].

Decreased concentrations of aldehydes may be attributed to the higher activity of ALDH in cancer cells [72, 79, 80]. For instance, overexpression of this enzyme has been reported in several tumor cells including non-small cell lung cancer (NSCLC) cell line [80] and esophageal cancer cells [81].

It has been speculated that the reason for a significantly decreased level of decanal in the headspace of lung cancer cells is related to mitochondrial defects in lung cancer cells which leads to a decreased level of ROS in the microenvironment of the cells and as a consequence, decreased lipid peroxidation [82]. On the contrary, an increase in the level of several aldehydes has been reported for several cancer types. The study of two main categories of lung cancers, namely small-cell lung cancer (SCLC) and NSCLC, has shown that the level of hexanal, as a general marker of oxidative stress, in the breath of SCLC patients is more than that of NSCLC patients. This can be partly explained by the higher activity of SCLC cells in terms of proliferation and metabolism compared to NSCLC cells [83, 84].

An increase in the level of hexanal and acetaldehyde has been identified in the headspace of the human promyelocytic leukemia cell line, HL60 [85]. It is known that human blood cells are able to metabolize ethanol to acetaldehyde [86, 87]. Neutrophils can oxidize amino acids and produce aldehydes [88]. Also, it has been suggested that generated ozone in neutrophils can react with cellular fatty acid resulting in the oxidation of omega-6 unsaturated fatty acids and the production of hexanal [85].

Levels of acetaldehyde may be modulated by a balance between its production of alcohol by ADHs and its elimination by ALDHs which change it to acetate. Therefore, the levels of acetaldehyde in biological matrices are closely related to the balance between ADHs and ALDHs activities and the metabolism of ethanol.

Due to the ability to form various types of DNA adducts, acetaldehyde has been classified as Class I carcinogen for humans. Acetaldehyde inhibits the enzymes involved in DNA repair leading to impaired DNA damage response. The link between acetaldehyde and several cancers like gastric cancer is evident [89].

Since tobacco smoke contains acetaldehyde, the high concentration of it in cancer patients may show a relationship between smoking and cancer. However, further investigation is needed to identify the exact role of aldehydes in cancer cell metabolism and their function in different types of cancer. It should be mentioned that gut flora can generate acetaldehyde from ethanol [90]. Formaldehyde and acetaldehyde are also present in the environment [91, 92].

It has been reported that the production of acetaldehyde is more efficient in the 3D culture models in comparison with 2D ones [93]. This could be due to the fact that 3D *in vitro* culture models mimic some properties of biological systems.

Benzaldehyde is another important aldehyde which is involved in several metabolic pathways, such as glycolysis/gluconeogenesis, tryptophan metabolism, and fatty acid metabolism [94].

Moreover, some aldehydes like nonanal have been considered biomarkers of apoptosis. It has been demonstrated that the levels of nonanal, 1,3-bis(1,1-dimethylethyl)-benzene, and 2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione significantly increase during apoptosis [95].

The general problems of analysis of aldehydes are their low concentrations in biological matrices and their high tendency to react with other compounds or breakdown during sample preparation or storage. However, high molecular weight aldehydes, like hexanal, heptanal, octanal, and nonanal are more stable than low molecular weight ones [96]. Fuchs *et al.* addressed this problem through the transformation of reactive aldehydes into stable oximes by means of on-fiber-derivatization [51]. Oximes are produced by the reaction of hydroxylamine with aldehydes or ketones. VOCs that have been reported as biomarkers for at least five types of cancers, namely hexanal, heptanal, octanal, and nonanal, are all high molecular weight aldehydes with weak polarity.

## Ketones

Production of ketones is also closely related to the higher oxidation rate of fatty acids that are observed in several cancers [24, 97]. The acetyl-CoA, which is a substrate for ketogenesis, is mainly formed as the major product of  $\beta$ -oxidation of long-chain fatty acids in mitochondria.  $\beta$ -oxidation of branched fatty acids (e.g. valproic acid) results in the formation of heavier ketones (3-heptanone) [98]. Ketones in biological matrices may also originate from exogenous sources like food or the ambient air pollution [55].

The ADHs are known to catalyze the oxidation of aliphatic alcohols to ketones, with a wide range of chain lengths. Although the primary alcohols are the most preferred substrates for ADHs, metabolism of secondary alcohols (e.g. 2-propanol and 2-octanol) to ketones (e.g. acetone and 2-octanone) by ADHs have also been reported. In several types of cancers, like liver cancer, it has been demonstrated that the ADHs activity in the affected tissues is significantly higher than healthy ones. Production of ketones in human hepatocellular carcinoma cells can be attributed to metabolism of long-chain secondary alcohols in culture medium by highly active ADHs in the cells [76].

Acetone, the simplest ketone, is derived from decarboxylation of acetoacetate and the dehydrogenation of isopropanol. Acetone is a product of the spontaneous breakdown of acetoacetate and gives a distinctive odor to the breath when exhaled by the lungs. In the human body, acetone is mainly produced by decarboxylation of acetoacetate which is formed by both lipolysis and the breakdown of ketogenic amino acids. Decarboxylation of acetoacetate to acetone may occur either in an enzyme-catalyzed way, by acetoacetate decarboxylase [99–101], or by nonenzymatic reactions [102].

The oxidation of isopropanol (2-propanol) is another source of acetone. Isopropanol is metabolized by ADH to form acetone [103]. ADH dominantly catalyzes oxido-reduction reactions between acetone and isopropanol.

Mitochondrial oxidation of fatty acids generates acetyl CoA that can enter the Krebs cycle. Cancer cells exhibit altered glucose metabolism known as the Warburg effect in which their energy production shifts from the Krebs cycle to glycolysis. Therefore, acetone and other ketone bodies (acetoacetate and  $\beta$ -hydroxybutyrate) are produced by the hepatocytes from excess acetyl-CoA which in turn results in an increased level of acetone in the body.

CYP2E1 plays an important role in the degradation of acetone through the conversion of acetone into acetol and acetone is also considered the physiological inducer for CYP2E1 [104, 105].

The evidence revealed that VOCs, like acetone, can be produced by a wide variety of anaerobic and aerobic bacteria [106]. It has been proposed that a considerable fraction of ketones in urine arise from bacterial action in the gut.

Since the concentration of acetone in bodily fluids or breath changes during some activities like fasting, exercising, and food consumption, acetone is not recommended by some researchers to be used as a biomarker [107, 108].

Cyclohexanone, another important cancer-related VOC, might be formed from the oxidation of cyclohexane. Different amounts of cyclohexanone have been found in exhaled breath of healthy and chronic obstructive pulmonary disease patients [76].

2-Nonanon, 3-heptanone, and 4-heptanone are three other ketones that are considered cancer biomarkers. 2-Nonanon can be produced from nonane metabolism by CYP450 [109–111].

The origin of 4-heptanone is still unknown. Previous studies have shown that 4-heptanone can be produced from the *in vivo* metabolism of plasticizers in the body [31, 112].

## Hydrocarbons

A potential source of saturated hydrocarbons (e.g. C<sub>3</sub>–C<sub>11</sub>) may be the lipid peroxidation process. However, this mechanism is probably irrelevant to the presence of branched hydrocarbons. The unmetabolized hydrocarbons excrete to the blood, and consequently to urine and/or breath. The concentrations of volatile hydrocarbons in biological matrices depend on their solubility in the different biological media. Hydrocarbons, with low solubility in the blood, instantly pass into the breath.

Alkanes are mainly formed during lipid peroxidation of PUFA constituents of biological membranes leading to the degradation of phospholipids and eventually cellular deterioration [52, 113]. Alkanes can be also produced in association with hepatic ethanol metabolism by ADHs.

The increased level of several alkanes (such as dodecane and pentane) and methylated alkane (e.g. 3-methylhexane) has been reported in the patients with different types of cancers [114–116]. Altered activity of CYP450 might be the reason for significant changes in the levels of alkanes and methylalkanes in patients suffering from cancer [117]. There is some controversy about the origin of methylated alkanes. It is thought to be secondary products of oxidative stress [118] by some researchers; however, others disagree with this hypothesis [119]. Isoprene (2-methyl-1,3-butadiene) can be formed by enzymatic or nonenzymatic pathways. Isoprene is produced along the mevalonate pathway of cholesterol synthesis [120]. Dimethylallyl pyrophosphate can be converted to isoprene nonenzymatically.

In human liver microsomes, CYP450 oxidizes isoprene mainly to 3,4-epoxy-3-methyl-1-butene, and 3,4-epoxy-2-methyl-1-butene, which are further hydrolyzed to vicinal diols (2-methyl-3-buten-1,2-diol and 3-methyl-3-buten-1,2-diol) [76].

A very low level of isoprene in gastric cancer tissue compared to a higher amount of that in healthy gastric tissue has been reported. This might be due to the ROS damaging effects happening in the gastric cancer tissue [121]. Also, isoprene is present in cigarette smoke [122] and can be considered as an exogenous compound. Some VOCs, like isoprene, can be stored in different tissues. Depending on the types of VOCs and the storage capacity of the tissue, the release time of VOCs varies. The amount of isoprene in the human body easily changes during exertion of a physical effort [123–126]. Even a few legs or arm contractions can lead to elevation of isoprene levels in exhaled breath [38]. It has been proposed that isoprene is stored in muscles and released from the working muscles through exercise [127].

### Aromatics

The origin of aromatic compounds like *p*-xylene is still unknown and they have been considered as possible environmental contaminants (e.g. cigarette smoke) in previous reports. For instance, benzene derivatives have been observed in the breath of patients with lung cancer. They are also found in the breath of smokers. Some researchers believe that as the amount of several aromatic compounds (such as benzene, toluene, and 2,5-dimethylfuran) increases in the breath of smokers versus non-smokers and lung cancer patients; they can be considered as tobacco-related carcinogens [24, 128].

### Alcohols

One of the main sources of alcohols in body fluids is diet. Alcohols from the ingestion of food and beverages are absorbed through the gastrointestinal tract and then released into the bloodstream. However, they can also be detected in feces, urine, breath, skin secretions, milk, and saliva [24].

In addition, alcohols can be produced from the metabolism of hydrocarbons. As mentioned earlier, the metabolism of alcohol is mainly catalyzed by ADHs [55, 129, 130]. In the cytosol of hepatocytes, ethanol is metabolized to acetaldehyde, a carcinogenic compound, by ADHs. Acetaldehyde is rapidly oxidized to acetate by ALDHs in the mitochondria. Acetate may be released to the blood or metabolized further to form CO<sub>2</sub>, H<sub>2</sub>O, or fatty acids as well as entering into intermediary metabolism as acetyl-CoA [130].

CYP450 isoenzymes are also involved in the oxidation of alcohols. CYP2E1, 3A4, and 1A2 are predominantly found in the ER, contribute to alcohol metabolism [130]. Generally, when the level of alcohol is elevated, CYP2E1 is induced, resulting in oxidation of the excessive amount of alcohol to acetaldehyde, and generation of ROS [130].

Obvious changes (decreases or increases) in levels of ethanol have been observed in several cancers including lung, liver, colorectal, and gastric cancers. Increasing evidence suggests that alcohol may induce carcinogenesis through aberrant DNA methylation [131]. PPAR- $\alpha$ , SREBP-1c, and PNPLA3 are some genes affected by chronic alcohol consumption [130]. The reduced levels of ethylhexanol in blood and breath of patients with papillary thyroid carcinoma and colorectal cancer, respectively, have been reported. This can be due to the consumption of ethylhexanol by the cells during tumor cell proliferation. Further investigations have identified the releasing of 2-ethyl-1-hexanol from NCI-H2087 lung cancer cell line [78].

The activity and expression of specific enzymes that regulate the metabolism of ethanol are influenced by genetic

polymorphisms [129]. Moreover, alcohol metabolism might be affected by a different amount of water and fat in the bodies of different people and different genders [55].

### Conclusion and future research

VOCs contain valuable information about biological processes inside the cells. The association between cancer and VOCs produced in the human body has attracted a lot of interest. Finding distinguishable VOC fingerprints or chemical groups related to cancers may lead to the early detection of cancers, the unraveling of the mechanisms of cancer development and progression, and eventually making the manipulation of the altered pathways possible. Cellular events or biochemical pathways associated with the cancer initiation and progression, such as the altered activity of the CYP450 system, can be translated into VOC profile. Therefore, analysis of VOCs in breath or body fluids has the potential to achieve cancer detection at very early stages. In addition, changes in signal transduction, gene regulation, and cellular proliferation can be followed by the VOC pattern of cells or organisms. VOCs enter into the blood system and are released later through breath, urine, feces, and skin. In contrast to the determination of most of the traditional biomarkers, measurement of exhaled VOC levels in breath is completely noninvasive and offers the potential for the development of screening tests and for disease monitoring.

Although significant efforts have been made by various research groups in the past decade to find new VOC cancer biomarkers, they have not been prospectively compared or analyzed in various cancers. Herein, we analyzed the COD database containing detailed information about cancer-related VOCs to identify significant VOCs associated with cancer and their origin.

Some of these compounds appear in more than one cancer, while some are unique compounds. Surveying these VOCs can be used to discriminate patients from healthy individuals. A comprehensive analysis of the COD database has revealed that a total of 18 VOCs from five major chemical compound categories are reported in various types of cancers and can be considered as important VOCs biomarkers. Highlighted VOCs includes six aldehydes (heptanal, hexanal, decanal, nonanal, pentanal, and octanal), four ketones (acetone, 3-heptanone, 2-butanone, and cyclohexanone), one alcohol (2-ethylhexanol), four hydrocarbons (dodecane, 3-methylhexane, 4-methyloctane, and 2,2-dimethyldecane), and three aromatic compounds (1,2,4-trimethylbenzene, 1-methyl-4-propan-2-ylbenzene, and *p*-xylene). There are several mechanisms for the production of hydrocarbons, aldehydes, aromatics, alcohols, and ketones which have been comprehensively reviewed in this article.

As discussed in this report, along with the benefits of VOCs as potential cancer biomarkers, there are some challenges for cancer diagnosis based on VOCs. The major problem is the strong effect of other parameters on VOCs pattern. VOCs can originate from both endogenous and exogenous sources. Food consumption, medications, physical activities, smoking, other noncancer diseases, and normal gut bacterial flora can all change the pattern of VOCs.

Another limitation arises from the fact that the sites and the origins of many VOCs are not clear. The information provided here is particularly useful for scientists investigating cancer-related VOCs, and for researchers who work on the development of sensors and electronic nose systems for cancer detection. However, there appear to be gaps in the studies that have been investigating VOCs as cancer biomarkers. Filling

these gaps might help to solve the puzzle of cancer-related volatile compounds. We hope this article will stimulate new quantitative experimental and theoretical studies of cancer-related VOCs and be valuable for improving current cancer researches.

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