Biomarker Assessment in Nutritional Modulation of Oxidative Stress-Induced Cancer Development by Lipid-Related Bioactive Molecules

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Abstract: Cancer, a leading cause of death, can be prevented by different nutrients, in accordance to epidemiological and experimental data. Cancer chemoprevention might involve different dietary substances, which can counteract genetic damage and modulate the acquisition of a neoplastic phenotype. Critical to this process is redox cellular homeostasis, with antioxidants and essential biomolecules being the most promising functional compounds of the diet. Nutritional interventions require accurate biomarkers in order to evaluate their appropriateness. Such parameters may be biological targets involved in the oncogenetic process, and biochemical changes deriving from the organic response to tumours, which can be considered as endpoints of dietary interventions. This review will thus focus on patents on recent progress in the development of redox-related anticancer nutritional interventions involving lipophilic compounds, and of biological markers for evaluating them, with their scientific basis being reviewed.

Keywords: Apoptosis, biomarker, cancer, chemoprevention, cytotoxicity, diet, fatty acid, flavonoid, free radical, lipid, nutrition, oxidative stress, polyphenol.

INTRODUCTION

There are many dietary compounds which are able to reduce the incidence and/or to control the evolution of oncological diseases [1], such as liposoluble biomolecules closely related to cellular redox homeostasis (e.g. fatty acids and polyphenols). Regarding this, we proposed the term *Onconutrition* to assess the biomedical role of nutrients as epigenetic modulating agents of the processes involved in cancer pathogenesis. This kind of approach enables different health strategies to be developed for preventing tumour appearance and progression [2]. In order to confirm this in humans, suitable biological parameters are required, among which the properties of transformed malignant cells and systemic responses to neoplastic disease might be used as biomarkers of cancer development. Regarding this, criteria to define a biomarker were taken from McCarthy and Shugart [3].

I. NUTRITIONAL CANCER CHEMOPREVENTION BY REDOX-REGULATION

Involvement of Oxidative Stress in Cancer Development

Cancer is a multistage process with increasing failures in the mechanisms involved in cellular differentiation and proliferation. Clones are then able to invade and colonize various tissues [4]. Its stages are successively as follows:

1. Initiation (a normal cell is transformed): rapid and irreparable damage occurs in the DNA.
2. Promotion (the initiated cell becomes pre-neoplastic): Over the years, the clone suffers various biological changes.
3. Progression (the pre-neoplastic cell become malignant): Finally, a neoplastic phenotype appears (self-promoted growth, inhibition and death resistance, unlimited replicating potential, angiogenic induction, and capacity for tissue invasion and metastasis) [5]. The complexity of this process is increased by the cellular heterotypic microenvironment created within the tumour, with both malignant sub-clones and normal connective cells conditioning illness evolution [6, 7].

In this regard, there is a close relation between oxidative stress and neoplastic transformation involving different altered biochemical pathways and apoptosis-resistant clone selection due to hostile extracellular conditions [8]. Oxidative stress involves a loss of balance between the positive and negative effects of free radicals, with all responses being aimed at resetting the cell basal state. Nonetheless, these responses acquire pro-neoplastic properties when they become persistent [9]. Furthermore, free radicals can induce the three oncogenetic stages (initiation, promotion and progression) by oxidative damage of crucial molecules, such as DNA. Finally, tumour cells can exhibit different oxidation-induced genetic abnormalities [10], as follows:

- Increased oncogene activity (e.g. tyrosinases, embryonic products) and/or loss of tumour-suppressor gene protein products (e.g. p53, apoptotic molecules) as a consequence of chromosomal aberrations.
- Unregulated production of growth factors (e.g. insulin-like growth factor 2), and tumour angiogenesis factors (e.g. haematopoietic growth factors) by alterations in DNA methylation patterns and genetic imprinting errors.

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- Genetic instability leading to progressive loss of regulated cell proliferation, increased invasiveness, and increased metastatic potential.
- Alteration in enzyme patterns (e.g. increased levels of enzymes involved in nucleic acid synthesis, antioxidant defence, and macromolecular lysis).

The most important free radicals derive from oxygen (reactive oxygen species -ROS-; e.g. superoxide anion, hydroxyl radical) and nitric oxide (reactive nitrogen species -RNS-; e.g. peroxynitrite), with their effects being mediated by enzymatic and non-enzymatic derivates that in the former are more strongly oxidizing than molecular oxygen itself. The formation of these intermediates must be steadily controlled by the cell in order to maintain a redox homeostasis, which is involved in mechanisms of cellular signalling, regulation and survival in tissues [11], with high amounts of these being deleterious [12].

Cancer Chemoprevention by Redox-Modulating Nutrients

The effects of reactive species will therefore depend on the extent to which their formation leads to homeostatic recovery (normal stress response) or to chronic deregulation and cellular transformation. Within this frame, nutrition plays an important role in these processes because different essential micronutrients and other dietary compounds are able to modulate free radical production beyond their own redox activity according to their bioavailability since [13], for example, the availability of a micronutrient does not necessarily follow increased consumption. Thus, dietary antioxidant compounds may act as cancer modulating agents, which can be general (for many cancer types) or specific (for a determined tumour) [14], and they has been thoroughly evaluated in cancer chemoprevention, such as lipids, trace elements (selenium, zinc), vitamins (A, C, E), thiols and polyphenols (flavonoids, curcumin), among others [15, 16]. Different compounds may be provided in foods, supplements, pharmaceutical compositions, cosmetics, or the like.

Cell susceptibility to nutrients depends on genetic information and epigenetic regulation, and the resultant phenotype may be modulated by nutrients. As a result, the expression of many genes is firmly regulated by the availability and quality of dietary compounds [17]. Moreover, the DNA damage caused by genotoxic and mutagenic insults (e.g. lipoperoxyl radicals) may be counteracted by cytoprotective agents found in foods and plants [18]. Another important aspect to be taken in account is the existence of nucleotide polymorphisms which determine human susceptibility to xenobiotic cancer modulators and potential pro-neoplastic phenotypes. Consequently, these polymorphisms must be also evaluated [19].

Among different nutrients, there two groups with relevant biomedical potential for cancer chemoprevention given their multi-target redox-related activity:

- **Unsaturated Fatty Acids:** The chemical composition of biological membranes can be manipulated by exogenous lipid intake to change their physiological properties [20]. The function of proteins associated with membrane rafts (e.g. some kinase species) will be compromised, with malignant parameters being affected. Thus, lipid integrity and membrane markers could be useful markers. In this regard, oxidative damage of polyunsaturated fatty acids causes the formation of lipid peroxides and other secondary products, which may deregulate cellular pathways and modulate the tumour redox resistance [21]. These metabolites have already been proposed as redox biomarkers in nutritional interventions [22], and their mechanisms of action were revised previously [14].
- **Polyphenols:** In recent years, a group of dietary compounds, the plant polyphenols, such as flavonoids, has been proposed for cancer prevention [23, 24], given their antioxidant activity and their affinity for biological membranes and other molecular targets [25, 26]. Also, they can change *in vivo* the bioavailability and the bioactivity of other substances, such as nutrients and drugs [27], which in turn can alter cell polyphenol activity [28]. Cancer chemopreventive and therapeutic methods based on the administration of polyphenol compositions have been recently developed after a various screening assays for their identification [29].

Also, both groups of bioactive molecules can interact in the organism given their lipophilicity. For example, triterpene saponins, constituents of many folk remedies and some of the more recently developed plant drugs, are known to form complexes with cholesterol by binding plasma lipids [30]. Thus, polyphenolic compounds might change the cellular response to lipid intake and, as a result, modify fatty acid bioactivity. Moreover, they are able to inhibit oxidative stress-sensitive pro-carcinogenetic pathways, such as the NF-κB activity [31], which is an ubiquitous transcription factor that regulates the transcription of a number of genes involved in immune and inflammatory pathways, such as pro-inflammatory cytokines and adhesion molecules, and in apoptosis, and thus is one of the central regulators of an organism's responses to various stress signals [32]. Deregulation of NF-κB contributes to a variety of pathological conditions such as septic shock, acute inflammation, viral replication, and some malignancies [33]. Because of its role in inflammation and carcinogenesis, it follows that down modulators of NF-κB would have tremendous therapeutic implications. Furthermore, some downstream effects due to inhibition of NF-κB activity are a decrease in levels of inducible nitric oxide synthase (iNOS, a lipid membrane-bound enzyme) and cyclooxygenase-2 (COX-2, an eicosanoid-related enzyme) expression. Both iNOS and COX-2 have critical roles in the response of tissues to oxidative injury, and carcinogenesis [34] Figs. (**1-2**).

Biological Effects on Neoplastic Cells

Basically, neoplastic cells have lost their differentiation grade to varying extents, but they have unlimitedly increased their proliferating capacity. Thus, biomedical approaches aim at inducing cytodifferentiation or cytotoxicity, respectively, and in this regard, nutrients can act as cytodifferentiating or cytotoxic agents in cancer therapy, whereas they

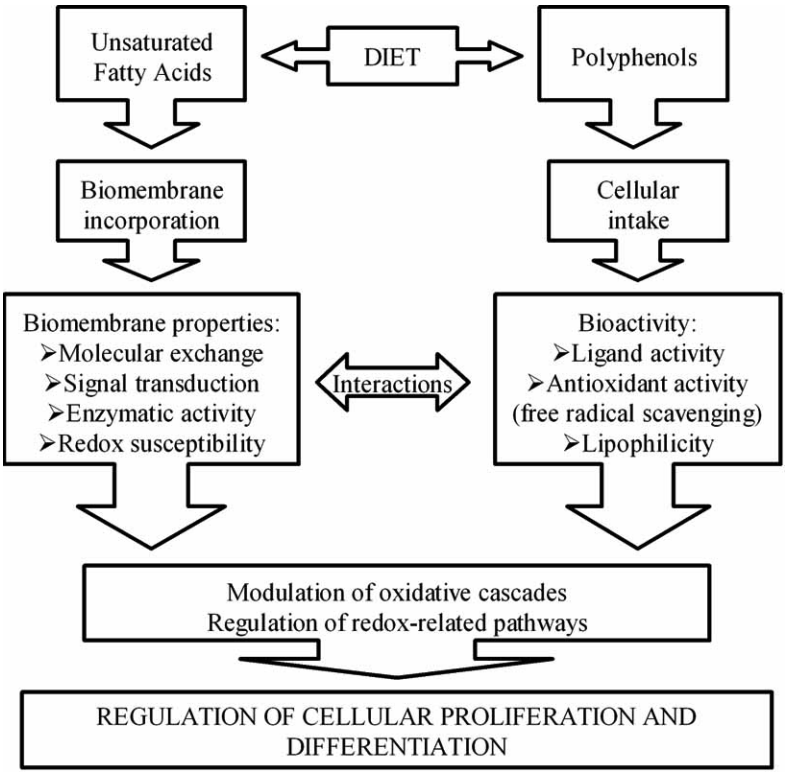

Fig. (1). Cellular regulation by dietary hydrophobic compounds.

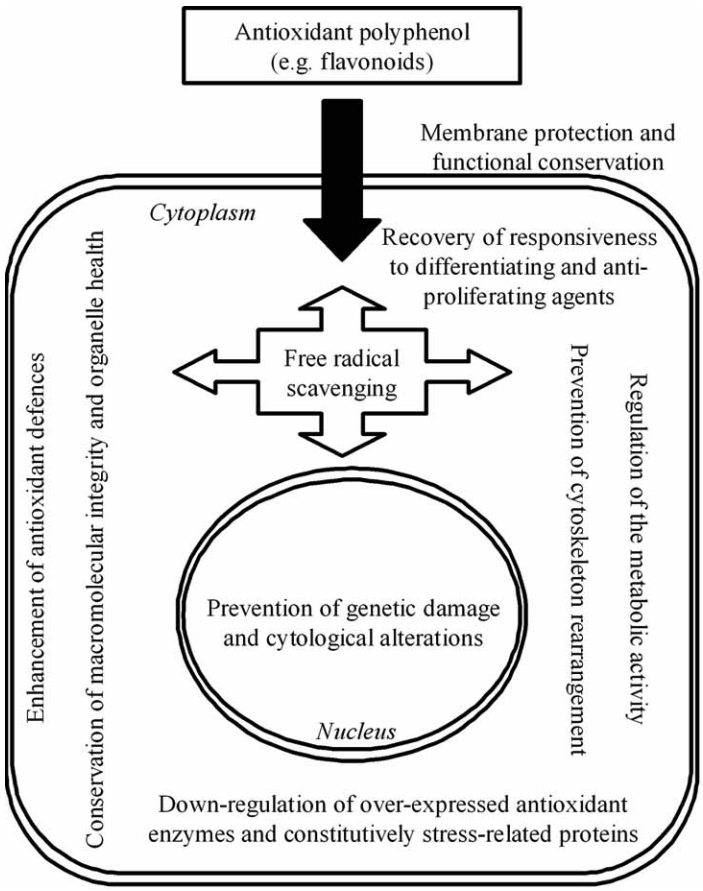

Fig. (2). Cancer chemopreventive redox-dependent effects of flavonoids.

should be cytoprotective for normal cells [35]. Cytoprotection and cytodifferentiation are thus crucial for cancer chemoprevention, and involve various actions intended to conserve or restore normal features in cells. For example, different regulators of the non-genomic action of progesterone have been developed in order to attenuate progesterone's inhibition of ovarian cancer apoptosis so that the neoplastic tissue recovers its responsiveness to therapeutic agents [36]. Among the polyphenols, 2-oxadiazolechromone derivatives are inhibitors of tyrosine kinase and can be employed for the treatment of tumours, for neuroprotection and for protection of the stress proteins of the skin [37].

When cells are initiated and acquire neoplastic characteristics, another kind of approach is necessary in which the induction of cytotoxicity in tumour cells is the main resource, although cytodifferentiation may still be involved in controlling some malignant properties [38]. Cytotoxicity involves the loss of cellular viability (cytolethality), the control of cellular proliferation (cytostasis), and pro-lethal injuries at different subcellular levels leading to cellular dysfunction (cytocompromise). Among different macromolecular targets, protein oxidative biomarkers may be any amino acid that has undergone oxidation (or other modification, e.g. chloro-tyrosine, dityrosine), and in this case especially, oxidized sulphur- or selenium-containing amino acids, which may be detected with an antibody that binds to them. The presence or amount of a biomarker present in a sample may aid in assessing the efficacy of environmental, nutritional and therapeutic interventions, among other uses [39]. The carcinogenetic involvement of redox metabolism has been confirmed by the fact that metal-associated compounds are able to modulate cancer progression. For example, amino acid sequence variants of cupredoxins are chemopreventive agents, with cancer death comprising cytotoxic variant endocytosis accomplished by the use of protein transduction domains derived from cupredoxins, including the p18 and p28 truncations of azurin [40]. Moreover, expression of antioxidant genes is one of the most important criteria, together with DNA repair and transcription factor genes, for assessing cancer risk, with the determination of transcript abundance as a suitable means [41].

The modulation of cell proliferation by a determined polyphenol composition (e.g. polyphenol compounds extracted from *Olea europaea*, Caiazzana olives) comprises the induction of cell cycle arrest and apoptosis [42]. Another example of an anticancer nutrient is genistein, a naturally occurring isoflavonoid isolated from soy products, which is a tyrosine kinase inhibitor which has been shown to inhibit the proliferation of oestrogen-positive and oestrogen-negative breast cancer cell lines [43]. Some plant triterpene aglycones have also been shown to have cytotoxic or cytostatic properties against human carcinoma cells [44].

II. BIOMARKER DEVELOPMENT

Clinical-Epidemiological Framework

The role of nutritional biomarkers was described in 1983 by Solomons and Allen, who emphasized their crucial role in the assessment of nutritional status [45]. In recent times, technological advances in bioengineering, molecular biology and physiology have enabled the development of different tests to identify parameters which directly reflect food consumption [46]. Thus, nutritional biomarkers have been developed to evaluate and validate different types of dietary surveys and interventions, and the future is promising because the methodologies applied have been progressively improved, reducing technical and analytical errors [47]. On the other hand, while biomarkers are frequently used as surrogate efficacy endpoints to expedite the process, biomarker assessment and validation has proven to be difficult because dietary agents exert multiple actions with an unknown hierarchy of biological importance [48]. A valid nutritional biomarker should have a specific, appropriate half-life and a good biomarker-intake correlation [49].

The use of biochemical markers for assessing nutritional status in clinical practice involves the identification of compounds [50], such as:

- Proteins: albumin, transferrin, retinol-bound protein, etc.
- Carbohydrates: glucose, etc.
- Lipids: cholesterol, triglycerides, etc.
- Vitamins: vitamin C, vitamin E, etc.
- Minerals: zinc, iron, copper, sodium, potassium, etc.

These molecules and elements have been studied extensively in risk assessment of different chronic illnesses (e.g. heart ischemia, dementia and cancer) [51, 52].

Blood is the most commonly used biological fluid for biomarker determination. Although some tests can be made in whole blood, plasma (serum) is more appropriate since it is more sensitive to dietary intake [53]. For example, plasma cholesterol levels are major cardiovascular risk markers, and its application extends to the study of various tumours, such as breast cancer [54]. Moreover, recent technical advances, such as the differential scanning calorimetry, allow blood proteomic profile to be assessed in cancer diagnosis [55]. Another sample is urine, which is a good indicator of the intake of hydrosoluble compounds, since their concentration in urine depends on the degree of saturation of nutrients in tissues [56]. Recently, the use of saliva for analysis of biological compounds to evaluate the nutritional status has expanded, as this is easily obtained by non-invasive methods and presents suitable levels of lipids (e.g. cholesterol, unsaturated fatty acids) and other dietary compounds [57].

One of the most significant advances is the development of biomarkers designed to decrease methodological inaccuracies in dietary studies, to be used as additional measures of environmental exposure and as tools to test the effects of diet in chronic diseases [58]. Such biomarkers emerge from the existing interactions between diet, lifestyle, genetics, and the risk of many chronic diseases [59], with nutritional epidemiology becoming a strong support for oncological approaches [60]. Nowadays, a combination of biomarker measurements with long-term dietary intake parameters is the preferred choice to measure exposure data [61]. This was the scientific rationale for designing and developing the European Prospective Investigation into Cancer and Nutrition (EPIC), which combines epidemiological and laboratory studies on healthy people, with the assessment of biochemical markers and genetic susceptibility [62]. Among the evaluated nutrients, fatty acids, carotenoids, tocopherols,

vitamin D, folate, vitamin B12 and trace elements were assayed in plasma [63].

For oncological risk assessment, genetic studies must be associated with specific biochemical activities to identify individuals with increased or decreased risk of some cancer types, involving the use of biochemical (e.g. hormones, enzymes) and genetic profiles of specific alleles of different genes [64]. For example, the methylation status of the COX-2 gene promoter region can be a prognostic biomarker of therapy [65]. This kind of approach has increased in risk evaluation given the development of molecular marker detection by RNA microarrays [66]. Nonetheless, cytological methods of detecting progression from pre-cancer to cancer are still valuable, utilizing toluidine blue staining as well as detecting allelic variation at microsatellite loci, with an allelic variation in one or more loci being indicative of that progression [67]. Between cellular parameters and molecular markers, ultrastructural biomarkers from a given biological sample can also be identified, with the image generated of ultrastructural biomarkers being subsequently used, among other things, for screening and monitoring diseases, evaluating drug and therapeutic efficacy, and assessing risks associated with a drug or therapeutic candidate [68].

Biochemical Assessment of Lipid-Related Biomarkers

The implications and effects of dietary compounds on oxide-reduction processes can be evaluated through several specific biomarkers. These indicators mainly include the oxidative products of lipids, proteins and nucleic acids induced by free radical activity, and the complex of antioxidant molecules which appears as a response [69]. As regards lipids, radicals attack compounds with the structural element -CH=CH-CH_2-CH=CH-. Thus, in theory, they may act on all polyunsaturated fatty acids (PUFAs), either in free or conjugated forms, although some PUFAs are better ROS substrates than others. Radicals induce the generation of peroxyl radicals, which oxidize a great variety of biological molecules including proteins and nucleic acids. In tumours, non-enzymatic lipoperoxidation processes can be induced artificially by the treatment of pure PUFAs with bivalent metal ions. Although there are other available methods, products are appropriately separable after appropriate derivatisation by gas chromatography and can be identified by electron impact mass spectrometry [70].

Lipids are thus one of the most important molecular groups involved in oxidative stress because they are sensitive to oxidation and are then able to initiate a chain reaction with other macromolecules [71]. They can also interact with several hydrophobic substances of the diet, such as various unsaturated fatty acids and polyphenols [72]. In oxidative lipid derivates, underlying mechanisms involve a sequence of reactions which are measurable. The presence of free radicals can be assayed in biological samples by different colorimetric tests [73]. Then, conjugated dienes (an upstream lipid oxidative state) can be obtained by a Fölch extraction followed by a UV-spectrophotometric assessment [74]. Although this parameter is classically used for lipid peroxide determination, the possibility cannot be discarded that an important proportion of conjugated dienes may be conjugated as linoleic acid, which can be incorporated by diet. Nonetheless, their *in vitro* levels may be attributed to changes in the redox membrane state, since mammalian cells are not able to synthesize that fatty acid [75]. This hypothesis is supported by the fact that directly quantified lipid hydroperoxides have a strong correlation with conjugated dienes in cells treated with arsenite and flavonoids [76]. Lastly, final derivates of lipid oxidation are short-chain peroxide compounds, such as OH-nonenal [77]. Although the presented biochemical markers can adequately reflect the lipid oxidation state, they have not been proposed as mandatory biomarkers of dietary intervention effects.

The lipid biomarkers chosen to be assessed in humans might include:

- Malondialdehyde (MDA): This is an end-product of lipid peroxidation normally present in urine and blood, and can be quantified directly by different methods, such as spectrophotometry, high performance liquid chromatography and gas chromatography with mass spectrometry [78]. Its urinary levels are associated with a person's whole redox state, and two colorimetric tests have been recently developed to measure MDA in this fluid. They are easily performed and involve fuchsin or pararosaniline HCl [79, 80]. Also, MDA levels can be measured spectrophotometricly by a reaction with thiobarbituric acid (TBARS assay), which are generally used as an indicator of lipid peroxide generation [81]. TBARS can be measured in tissues, but are generally measured in plasma [82].
- Oxidized low density lipoprotein (LDL-ox): Oxidative damage to cholesterol-carrying molecules has been hypothesized to be a causative agent in the development of various pathologies, such as cardiovascular diseases [83] and certain types of cancer [84, 85]. LDL-ox forms a complex with β2-GPI within the body. This should be measured preferably in blood, and the resulting product from an immune reaction is assayed spectrophotometrically [86]. This technique enables various human diseases to be detected [87].
- F2 isoprostanes (F2-iso): These compounds are a family of prostaglandin F2-like compounds, which are formed by free radical-catalyzed peroxidation of arachidonic acid [88]. The most abundant F2 isoprostane is 8-isoprostaglandin F2a (8-iso-PGF2a), which has been suggested to be a promising marker for oxidative injury [89]. Isoprostanes may be measured in a plurality of human body fluids such as urine, blood, tears and saliva, but urine is generally considered the best matrix for quantifying isoprostane levels *in vivo* [90], with immunoassays or gas chromatography/mass spectrometry being generally used [91, 92].
- Breath hydrocarbons (BHC): Carcinogenesis is accompanied by increased production of ROS which degrade membranes by lipid peroxidation. The process involves the formation of volatile organic compounds, which are excreted in the breath. Volatile oxidation products of n-3 and n-6 fatty acids, such as ethane and pentane, are commonly used. This is a non-invasive approach for estimating lipid peroxidation *in vivo* [93]. BHC concentrations are quantifiable by solid phase micro-

extraction and gas chromatography/mass spectrography techniques [94], or by electronic sensors [95].

- Hydroperoxides (HP): In order to study lipid peroxidation in human pathology, there is a simple and sensitive method for direct hydroperoxide measurement, the ferrous xylenol orange (FOX) assay, which has been previously validated and probed to be better than other techniques to measure hydroperoxide per se [96]. Moreover, FOX assay has been efficient for the measurement of 5-lipoxygenase activity at the stage of primary anticancer inhibitor screening [97]. Furthermore, they might use as cancer biomarkers when are increased in urine [98]. Recently, the method was automated to improve stability and accuracy, getting a precision value lower than 3% [99]. Also, the reactivity between HP and ferrous ion can be revealed accurately by chemoluminiscence [100]. The assessment of HP is very relevant given their widespread presence in different samples, such as tissues, cells and biological fluids [101], including within plasma LDL-ox molecules [102].

Although the list of potential lipid biomarkers has been increased [103], data which validate their predictive use in human cancer is really scarce. For example, predictive assessments of lung cancer, the most frequent tumour, mainly derive from genomics and proteomics [104]. Thus, there are a small number of lipidomic markers [105] Table **1**.

Table 1. Comparison of Lipid Biomarker Accuracy in Lung Cancer*

Biomarker (method)	Se	Sp	PV+	PV-
Plasma MDA (spectrophotometry) (a)	34%	76%	42%	70%
Platelet LA (gas chromatography) (b)	100%	76%	75%	86%
Breath HC (electronic nose) (c)	71.4%	91.9%	66.6%	93.4%

Abbreviations: HC: hydrocarbons; LA: linoleic acid; MDA: malondialdehyde; PV: predictive value; Se: sensitivity; Sp: specificity. References: a: 105; b: 109; c: 95.
*Different mentioned parameters are representative but non-limiting examples.

For the prevention of lipid oxidation, polyphenolic antioxidant activity may depend on the lipophilicity of these compounds. Indeed, quercetin is a better antioxidant agent against lipid hydroperoxide overproduction than counteracting aqueous hydroperoxide increase in cells [76]. Moreover, flavonoids are more effective antioxidants than other commonly used substances, such as selenium, when oxidative lipid damage is assayed [106]. It is also important to keep in mind that aqueous and lipid hydroperoxides have different generation kinetics [22]. This is very relevant due to the participation of these lipid metabolites in oxidative chain reactions and their role as ligands of cellular receptors involved in cell proliferation and differentiation [107]. Furthermore, they co-modulate other cancer-related pathways which can be regulated by nutrients. For example, eicosanoid binding to peroxisome proliferating activating receptors is a key point of regulation for epigenetic actions of different liposoluble compounds on cancer development, such as vitamin A [14].

Nonetheless, it is important to keep in mind that biomarkers are surrogate endpoints, i.e. measures of effect of a certain treatment that may correlate with a real clinical endpoint, but they do not necessarily possess a guaranteed relationship [108]. Another analytical option is the direct measurement of polyphenolic compounds (or metabolites of) in human specimens (bioavailability), although it reflects exposure but not effect. In this concern, there are efficient validated immunoassays for phytoestrogen determination in epidemiological studies. This is important given the close relation between phytoestrogen intake and the risk of breast and prostate cancers [61]. In addition, fatty acids themselves might be used as tumour biomarkers. Chromatographic lipid profile of erythrocytes and platelets from non-small cell lung cancer patients have revealed that some fatty acids are related to this cancer type (22:0 and 18:2n6 in erythrocytes, and 16:0, 18:0, and 18:2n6 in platelets), with platelet linoleic acid (18:2n6) being the best biomarker [109]. Also, both cell types showed essential PUFA decrease, which are correlated with the illness and with their susceptibility to oxidative stress with changes in the fluorescence of lipid extracts [110].

CURRENT & FUTURE DEVELOPMENTS

In order to discover new anticancer agents, it is essential the assessment of different biological parameters, called biomarkers, which allow evaluating the effectiveness of the use of such biomolecules, after the identification of their potential bioactivities. Thus, onconutritional researchers suggest that dietary compounds and their chemical derivates might be used as anti-tumour compounds, mainly in cancer chemoprevention. In view of these concerns surrounding the use of nutrients and derivates as anticancer agents, organic redox-modulating substances, such as unsaturated fatty acids (redox-sensitive linear molecules or chains) and polyphenols (redox-modulating cyclic molecules or rings) are promising given the very relevant involvement of oxidative stress in oncogenesis due to their physical-chemical properties. Specifically, polyphenolic compounds, such as antioxidant flavonoids, might be considered, since they possess multi-target and multi-stage actions, and they can reciprocally interact with lipids to regulate redox homeostasis and cell biology. Those may well constitute the only class of molecules which can modulate every stage of cancer development with a wide spectrum of regulatory activities.

Concerning translational medicine (the translation of laboratory-based research into clinical practice to diagnose and treat patients), this review has highlighted the rationale for the use in monitoring the effects of dietary intervention, mainly by antioxidant polyphenols, on oxidative stress-induced oncogenesis of experimentally established biomarkers as predictors of cancer nutritional modulation. Among them, MDA and TBARS are measurable by simple available techniques in organic fluids and tissues, and both represent the whole body status of lipid peroxidation. Nonetheless, they do not necessarily represent the nutritional status. On the other hand, LDL-ox does represent oxidative and nutritional status, since it depends on lipid and protein intake. Concerning F2-iso, they allow enzymatic lipid oxidation to be assessed, and they are also related to cancer-regulating pathways (eicosanoids). But, assays for its

determination are not already available in every clinical laboratory. This might be excelled by the inclusion of HP quantification, given its technical efficiency. Finally, given the promotion of non-invasive diagnostic methods, breath constitutes an incipient option, together saliva, to evaluate lipid oxidation by-products *in vivo*, such as the BHC. Beyond the promising development of lipid biomarkers, further studies are required to validate their utilization in human cancer monitoring.

Lipid molecules might be utilized as nutritional biomarkers of the polyphenolic bioactivity, since they are sensitive to oxidation, of which derivates modify cell functions and viability, and they are closely related to polyphenol effects on both normal (cytoprotection) and neoplastic cells (cytodifferentiation and cytotoxicity). Also, lipid oxidative products might be obtained from common biological samples, such as blood and urine. Furthermore, saliva has been recently recommended to be assayed for cancer monitoring (diagnosis and survey). Finally, biomarkers derived from biochemical studies should be developed into a clinical-epidemiological framework, with appropriate statistical techniques being required for outcome analysis. Thus, further long-term prospective studies in humans are required. Certainly, nutritional biomarker assessment and validation has proven difficult because dietary agents exert multiple actions with biological importance.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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