Food and *omics*: unraveling the role of food in breast cancer development

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Breast cancer is the second most common cancer worldwide and the most common cause of cancer death for women. Its plasticity and variability suggest a multifactorial origin, with powerful influence of environmental factors. Current scientific evidence pinpoints food and specific nutrients as crucial factors in breast tumor development. More precisely, dietary components can actively participate in the suppression and/or progression of cancer by introducing modifications into the epigenetic landscapes of cancer. Food not only can target oncogenes and tumor-suppressor genes and modify their methylation levels, but they also can influence histone chemical modifications, non-coding RNA pathways and microbiota metabolism. Breast cancer is currently treated with surgery, radiotherapy, chemotherapy and/or therapies targeting estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). However, the holistic omics study of the association between diet and breast health opens an interesting alternative for future breast cancer prevention and therapy.

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Introduction

Cancer is a leading cause of death worldwide, only outranked by cardiovascular diseases. As defined by the OMS (https://www.who.int/en/news-room/fact-sheets/detail/cancer), this is a generic term for a large group of diseases that can affect any part of the body, and often referred to as neoplasms or malignant tumors. Under this abnormal situation, the body's cells begin to divide and grow without control and old or damaged cells survive when they should die. When cells start to proliferate uncontrollably, these cells may form a solid mass called a tumor (also known as a neoplasm). Tumors can be cancerous (malignant) or non-cancerous (benign), and the main difference is the ability of the first ones to spread into, or invade, nearby tissues and other areas of the body. The latter process is referred to as metastasizing, and metastases are a major cause of death from cancer.

Cancer development involves genome mutations that originate both oncogenes and tumor suppressor genes, and scientific evidence suggests that both the gain of function of the first and the loss of function of the second are required for the disease to occur. In this dynamic context, a small number of traits are shared by most (and perhaps all) types of human cancer. As such, six essential alterations in cell physiology were proposed in 2000 by Hanahan and Weinberg as dictators of malignant cell growth, referred to as "cancer hallmarks" [1]: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These six biological capabilities are constant in different types of cancer, but are acquired through different mechanisms, and in different chronological order [1]. In 2011, Hanahan and Weinberg added two hallmarks to the list, *i.e.* reprogramming of energy metabolism and cancer capability of evading immune destruction [2]. Authors also defined what is called "tumor microenvironment", another dimension of complexity in this disease. Underlying these eight core hallmarks are genome instability and mutation, which generates the genetic diversity that expedites their acquisition, and tumor-promoting inflammation, which fosters multiple hallmark functions.

According to the Global Cancer Observatory (https://gco.iarc.fr/), the most common cancer worldwide is lung cancer, closely followed by breast and colorectal cancers. Overall, the most common cause of cancer death is lung cancer, but for women, breast cancer is the most diagnosed cancer and the leading cause of cancer-related deaths, worldwide. This breast disease is the most frequently diagnosed female cancer in most countries, including both higher and lower-income areas [3]. The WHO estimates that by 2040 (https://gco.iarc.fr/), there will be more than 3 million new cases of breast cancer each year in the world. It is widely acknowledged that cancer arises due to the interaction of genetic factors and external agents, including physical (e.g. UV radiation),

chemical (e.g. tobacco) and biological (e.g. viruses) factors. In breast cancer, age, mammographic density, nulliparity or late age at first parity, late menopause, alcohol intake, overweight, late menopause and exogenous hormone intake (contraceptive use and replacement therapies), are all well-established risk factors. On the contrary, young age at first delivery, physical activity and lactation have been related to a reduced risk of breast cancer. However, increased incidence rates in places where rates have been historically relatively low potentially reflect a combination of demographic and environmental factors, predominantly in postmenopausal breast cancer.

The breast is composed of three different types of tissues, *i.e.* glandular tissue (milk lobules and ducts), stromal/supporting tissue (fat and connective tissue) and lymphatic network. Breast carcinomas are a heterogeneous group of lesions, comprising a wide range of histological and biological variations, along with very distinct clinical courses. Based on the genes a tumor expresses, five main molecular subtypes of breast cancer have been defined: luminal A, luminal B (two subtypes), triple-negative/basal-like, and human epidermal growth factor receptor 2 (HER2)-overexpressing [4,5]. Both luminal types are estrogen receptor (ER)-positive, but luminal A is HER2 negative while luminal B can be either HER2 positive or negative. The prognosis is usually worse for luminal B breast tumors. Basal-like (triple negative) do not generally express hormone receptors (estrogen-receptor, ER, or progesterone-receptor, PR) or HER2, and HER2-enriched only expresses HER2. Additionally, Ki67 is the

factor that shows the proliferative activity of tumor cells and is used also for molecular classification [4,5]. The most frequent type is Luminal A and is also the one with the best prognosis. According to site, breast cancer can be classified as non-invasive or invasive, with the first one confined to the ducts (ductal and lobular carcinomas *in situ*) and the second invading the surrounding fatty and connective tissues (infiltrating carcinomas) from the milk lobules or ducts of the breast [4]. Other less commonly occurring breast tumors are medullar carcinoma, tubular carcinoma, or inflammatory breast cancer, amongst others.

Despite the multitude of molecular pathways involved in breast cancer development, the abnormal behavior of most tumors can be summarized into the eight phenotypic Hallmarks of Cancer, as previously defined [2]. However, substantial variations are noticed within and across countries, depending on the degree of economic development and associated social and lifestyle factors [3]. Both the plasticity of cancer patterns and its variation around the world implicate environmental factors as powerful determinants of its advent [6]. In particular, there is considerable evidence to implicate food and specific nutrients as key factors in breast cancer development. For example, the amount of fat intake after breast cancer diagnosis and the adherence to a high-quality diet, before and after diagnosis, have been related to the risk of recurrence and of death from other causes [7]. Alternatively, the increased consumption of soy products has been related to a decreased risk of recurrence and/or mortality of this type of cancer, particularly among

Figure 1
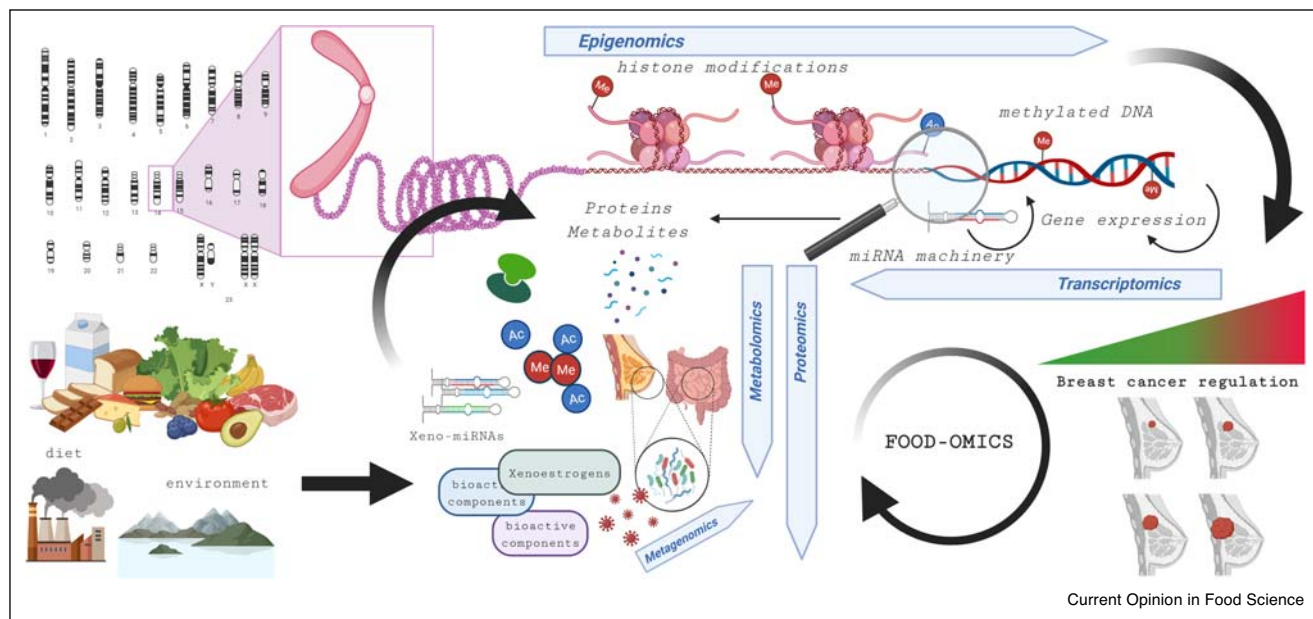
The impact of food and nutrients on breast cancer development can be characterized by multiple omics technologies applied at different levels of tumorigenesis. Created with BioRender.com.

Chinese women, with diverse molecular mechanisms involved in this chemo-protective effect [8]. In this sense, the anti-oncogenic properties of diverse phytochemicals on breast tissues have been widely recognized [9–13].

Recent advances in 'omics' technologies have led to breakthrough research in the characterization of molecular changes underlying the development of many human diseases, including cancer [14]. Genomics, transcriptomics, epigenomics, proteomics and metabolomics, amongst others, have greatly contributed to medicine, revealing several key mechanisms on tumor development and treatment. More precisely, omics have extended the knowledge on gene-diet interactions and laid the foundations for what is known as 'personalized' or 'precision' nutrition [15]. In this context, either the protective or the promoting effect of dietary components on breast cancer may be orchestrated by diverse mechanisms (Figure 1), with the latest scientific evidence notably pointing towards epigenetic ones as key players in this disease [10–12,16–18]. In this review, the most relevant 'omics' studies on the relationship between diet and breast cancer, published in the period from 2018 to present, are presented. An overview of some of the most outstanding and illustrative omics works in this field is provided in Table 1.

Food as epigenetic driver in breast cancer

Scientific evidence has demonstrated that genetic alterations and acquired epigenetic abnormalities co-participate to cause aberrant gene function or expression that are key features in cancer [19,20]. In this sense, the epigenome may be defined as the complete record of all the chemical modifications of DNA that regulate the expression of genes and are heritable without affecting the sequence of genome. These modifications bring different phenotypes, contributing to human diversity and evolution. Various studies have suggested a role for dietary compounds as dynamic epigenetic modifiers of breast cancer risk [12,21]. Besides, there are important variations in the epigenotype across breast tumor subtypes and hence the potential response to food compounds would be different too. Beyond genomics, genome-wide analyses of epigenetic marks (*i.e.* epigenomics) are expanding the understanding of cancer-diet interactions and providing new alternatives for diagnosis, prognosis and therapy [22]. Epigenetics mechanisms during oncogenesis include DNA methylation, histone modifications, chromatin remodeling and non-coding RNAs (mainly miRNAs) regulation. These epigenetic effectors in conjunction with genetic/chromatin lesions are responsible for altered gene regulation, activation of oncogenes and silencing of tumor suppressor genes in breast cancer [19,20] and they are even implicated in drug resistance/sensitivity [22,23]. A list of cutting-edge technologies used in breast cancer epigenomics research are provided and further discussed in a review by Davalos *et al.*

published in 2017 [22], along with a list of epigenetically regulated genes. Unlike mutations, epigenetic traits are reversible and open the door to new drugs (epi-drugs) or phytochemicals use for breast cancer treatment [24].

DNA methylome

It is suggested that diet can affect the DNA methylation by different mechanisms, including substrates and cofactors alterations, and/or changing the activity of various implicated enzymes such as DNA methyltransferases (DNMTs) [24,25]. Yet, the extent to which food influences methylation is unclear. A key advancement in this field resulted from the demonstration that sodium bisulfite could convert cytosine into uracil, while methylated cytosine will remain unmodified. Thanks to DNA bisulfite treatment, next generation sequencing (NGS) technologies have allowed the generation of comprehensive DNA-methylation profiles (*methylomes*) by measuring genome-wide 5-methylcytosine nucleotides, at single nucleotide resolution. However, the usually high costs of these technologies continue to limit their application. In connection with this, high-throughput methylation arrays have enabled quantitative interrogation of selected methylation sites, minimizing costs per sample and being more affordable for large sample populations such as those used in genome-wide association study (GWAS) cohorts. Methylation sequencing covers millions of methylation sites, while methylation arrays usually profile around half a million to one million of them. This phenomenon was found to target predominantly cytosine-phosphate-guanine dinucleotides (CpG), and it is referred to as CpG methylation. In breast cancer cells, some gene promoters are hypermethylated at their CpG-islands leading to inactivation of their expression by changing open euchromatic conformation to compact heterochromatic conformation [24].

The potential of the association between DNA methylation marks and breast cancer risk is supported by the many studies published on the topic. In this sense, it has been suggested that methylation profiles start to change in invasive breast cancer years before the tumor is even clinically detected [26], and that methylation marks can be applied in prediction models and survival analysis [27••,28]. Also, the resistance of ER-positive breast cancer to endocrine therapy such as tamoxifen or aromatase inhibitors has been associated to individual and multivariable DNA methylation markers, independently of luminal status [29]. These results suggest that resistance can be identified prior endocrine treatment. In a genome-wide study performed in 2011 by Fang *et al.* with almost two hundred breast tumor tissue samples, a signature was found in the methylome that may predict metastasis. Interestingly enough, the signature was valid independently of other breast cancer markers [30].

Table 1

Summary and description of the main *omics* strategies used for elucidating the role of food and nutrients in the development of breast cancer

Human Sample	Food component	Effects on breast cancer	Omics approach and technology	Reference
Cell culture: human mammary epithelial MCF10A cell line, human breast cancer MCF10CA1h and MCF10CA1a cell lines	Stilbenoids (resveratrol)	Hypomethylation (epigenetic reactivation of genes suppressing cancer)	*DNA methylomics* (genome-wide DNA methylation analysis); Illumina Infinium Human Methylation 450 K BeadChip microarray	Beetch *et al.* 2019 [35]
Peripheral blood	Polybrominated biphenyls (PBB)	1890 CpG sites associated to PBB levels	*DNA methylomics* (genome-wide DNA methylation analysis); Illumina Infinium MethylationEPIC microarray	Curtis *et al.* 2019 [41]
Transgenic mouse models: breast tissue	Broccoli sprout (sulforaphane)	Gene expression changes in multiple epigenetic-controlled tumor-specific genes (preventive effect)	Multi-omics (histone and DNA	Li *et al.* 2018 [16]
MCF-7 breast cancer cells	Ginseng (ginsenoside Rg3)	Cell growth inhibition	*DNA methylomics* (genome-wide DNA methylation analysis)	Ham *et al.* 2018 [36]
Plasma (extracellular vesicles)	Mediterranean Diet	Up and down-regulation of miRNAS	miRNA profiling; NanoString human miRNA panel	Kwon *et al.* 2020 [51]
MCF-7 and MDA-MB-231 breast cancer cells	Anacardic acid	anti-proliferative and pro-apoptotic activity	Transcriptomics; RNA-seq	Schultz *et al.* 2018 [57•]
Murine triple-negative breast cancer cell lines	Folic acid	type I interferon signaling modification	Transcriptomics; microarrays	Kok *et al.* 2020 [59]
MCF-7 breast cancer cell line	Xanthohumol C	modifications in cell-cell adhesion, cell cycles, DNA replication and type I interferon signaling	Proteomics; nano-LC HRMS	Roehrer *et al.* 2019 [60]
MCF-7, SKBR-3, and MDA-MB231 breas cancer cell lines	2′-Hydroxyflavanone	changes in the proteins responsible for BC incidence, metastases and therapeutic sensitivity	Proteomics; nano-LC HRMS	Nagaprashantha *et al.* 2019 [61]
MCF-7 breast cancer cells	Broccoli (sulforaphane)	Differentially methylated genes and differentially expressed proteins and metabolites; reversion of estradiol effects	Multi-omics (DNA methylomics, proteomics and metabolomics); Ilumina Infinium Methylation 850 K BeadChip, LC-MS and GC-MS in full scan mode	Huang et al 2020 [37••]
Breast tissue, urine, plasma	Polyphenols and methylxanthines	Chemoprevention	metabolomics; UHPLC-ESI-QTOF-MS	Ávila-Gálvez *et al.* 2019 [66]
Plasma	Dietary pattern (Western diet, alcohol, coffee)	Discriminant metabolites	metabolomics; untargeted LC-MS	Lécuyer *et al.* 2020 [64]
MCF-7 and MDA-MB-231 cells	Isoflavones	Modification of various cancer-related molecular pathways	proteomics; nano-LC UDMS [E]	Ilieş *et al.* 2020 [62]
MCF-7 and T-47D cells	Xenoestrogens (genistein, zearalenone)	Modified response to palbociclib and letrozole drugs	metabolomics; HPLC-QTOF-MS	Warth *et al.* 2018 [63]
Stool	Dietary fiber	Gut microbiota that are linked with β-glucuronidase activity modifies estradiol levels	metagenomics; 16S rRNA sequencing	Zengul *et al.* 2020 [75]

LC-MS: liquid chromatography coupled to mass spectrometry; GC-MS: gas chromatography coupled to mass spectrometry; UHPLC-ESI-QTOF-MS: ultra-high performance liquid chromatography coupled with electrospray ionization-quadrupole-time of flight-mass spectrometry; HRMS: high resolution mass spectrometry; nano-LC UDMS [E]: nano flow liquid chromatography coupled to ultra definition mass spectrometry.

Very recently in a Spanish study in 2020, Lorenzo *et al.* showed higher levels of *ZNF577* methylation in women with greater adherence to the Mediterranean diet or specific foods such as vegetables and fish. *ZNF577* methylation level has been previously highlighted as a possible epigenetic mark of obesity-related breast cancer [31]. Since the Mediterranean diet has been proposed as a beneficial dietary pattern on the promotion of health, as illustrated by previous similar studies implying different methylation sites [32], the findings by Lorenzo *et al.* could be considered counterintuitive and require further investigation. In a recent epigenome-wide association study, Do *et al.* [33] assessed the relationship between diet quality (Alternative Healthy Eating Index 2010) and blood DNA methylation, using the Illumina Infinium HumanMethylation450 Beadchip (Illumina Inc.), which enables assessing >450k methylation sites in a single analysis. Their results, with a discovery cohort of 4355 women and a replication cohort of 571 mono and dizygotic twins, revealed 24 DNA methylated CpG differentially associated to adiposity, inflammation and disglycaemia. As previously discussed, obesity and inflammation have been related to multiple cancer hallmark functions [2,31]. Beyond the importance of overall diet quality, certain plant dietary compounds have been proposed as anti-cancer molecules. The knowledge on the direct mechanisms for their beneficial effects in breast cancer is very limited, however, scientific evidence specifically points towards DNA methylation patterns [34]. This is the case of resveratrol and pterostilbene, natural components of different berries such as grapes or blueberries. A recent omics study by Beetch *et al.* using breast cell culture indicated that resveratrol often targets for methylation of the same genes but at different CpG *loci*, same gene families or genes of the same functional categories [35]. One of the strongest hypomethylation upon stilbenoid exposure was located in *SEMA3A*, a gene with recognized tumor-suppression potential in breast cancer. Similarly, a genome-wide methylation study by Ham *et al.* [36] identified over two hundred genes with significant changes at specific CpG sites in MCF-7 breast cancer cells treated with ginsenoside Rg3, a steroidal saponin metabolite of ginseng. Also sulforaphane (SFN), a sulfur-rich compound commonly found in cruciferous vegetables, could restore the changes induced by estradiol in the methylation levels of MCF-7 ER positive human breast cancer cells, reversing its adverse effects [37••].

Apart from natural components, food may also carry contaminants or food-contact materials that unintentionally pass from the environment to food during its production. As an example, bisphenol A (BPA) is a monomer of polycarbonate plastics that may be present in canned food and bottled beverages as a result of its migration from the lining of the packaging. BPA is considered an estrogen-like endocrine disruptor, and it has been associated with the development of breast cancer [38,39]. In 2019, Awada *et al.* performed a whole-genome DNA methylation profiling of bisphenol-exposed breast cancer cells, using the Infinium MethylationEPIC microarray from Ilumina Inc. (San Diego, CA, USA) that covers over 850,000 CpG sites, at single-nucleotide resolution [40••]. Despite its limitations, this pioneer study by Awada *et al.* showed that BPA has a strong effect on the DNA methylome of breast cancer cells, disrupting several cancer-related genomic clusters of or single CpG sites. In a similar study published in 2019, the DNA methylome of peripheral blood collected from almost seven hundred participants of the Michigan Polybrominated Biphenyl (PBB) registry was investigated using the abovementioned genome-wide methylation profiling microarray [41]. The Michigan PBB registry was recruited after an agricultural accident in the 1970's introduced PBB into the food supply of Michigan. This cohort reported many health concerns, including breast cancer, digestive cancer, and lymphomas, after being exposed *in utero* or in their childhood. The results obtained by Curtis *et al.* [41] indicated that exposure to PBB through food is associated with epigenetic marks and, like other endocrine disrupting compounds, it acts similarly to estrogens. Also, persistent organic pollutants (POPs), which persist in the environment and can bioaccumulate through the food chain, may lead to increased risk of developing breast cancer [42]. In this regard, a case-control study performed with 74 Greenlandic breast cancer cases and eighty controls found positive associations between serum POP levels and methylation levels, in particular for estrogen receptor genes *ESR1* and *ESR2* and circadian gene *PER1* [43].

Histone modification profiling

Gene dysregulation related to breast tumors can be caused by histone modification, including mostly acetylation and methylation of the amino acid tails of the histone proteins [44]. This epigenetic driver modulates the structure of chromatin and subsequently alters the accessibility of DNA. Major histone modification enzymes are histone acetyltransferases (HAT), histone deacetylases (HDACs) and DNA methyltransferases (DNMTs). A recent review by Klein and Hainer discusses techniques for determining DNA accessibility and nucleosome positioning, and alternatives for detecting chromatin-bound proteins [45]. ChIP-seq (chromatin immunoprecipitation followed by sequencing) is the most common profiling technique to assess chromatin-binding proteins, with numerous protocols and comparative datasets available. A high-throughput droplet microfluidics platform to profile chromatin landscapes of thousands of cells at single-cell resolution was recently proposed by Grosselin *et al.* [46], reveling a common chromatin signature in a subset of breast cancer cells resistant to drug therapy.

The influence of broccoli sulforaphane in global histone H3K9 and H3K14 acetylation levels were measured in study performed by Li *et al.* [16] using transgenic mouse models. Sulforaphane has demonstrated ability to target histone acetylases and DNA methyltransferases. More specifically, a broccoli sprout diet (sulforaphane) in a prenatal scenario exhibited preventive effects in breast cancer, primarily increasing histone acetylation rather than DNA methylation. However, the broccoli diet did not reduce mammary tumorigenesis when administered during adulthood, supporting the idea of a positive effect of cruciferous vegetables in early embryonic over breast health later in life. The existing literature on histone modifications in breast cancer is scarce, and even more its link with food. Nonetheless, the Lonestar Oncology Network for EpigeneticS Therapy And Research (LONESTAR) consortium provided a unique resource of subtype-specific chromatin signatures for breast cancer researchers, and revealed new insights into breast cancer biology [47]. The consortium was created to define epigenetic factors associated with molecular changes in specific subtypes of breast cancer.

Posttranscriptional regulators: miRNAs

MicroRNA (miRNA, miR) are a class of non-coding RNA molecules of approximately 20-30 nucleotides that regulate gene expression at post-transcriptional level. These molecules can regulate multiple genes by stimulating or degrading mRNA targets. Their role in breast cancer pathogenesis has not been fully elucidated yet but diverse oncogenic, tumor-suppressive and metastasis influencing miRNAs have been proposed so far [48,49]. Due to their stability, miRNAs may be advantageously studied in non-invasive samples, such as blood, serum and urine, and could serve as potential prognostic biomarkers.

Food can contribute to this epigenetic pathway through two fundamental routes: *1)* dietary components capable of altering miRNA expression, and *2)* dietary miRNAs codified by non-human genomes (xeno-miRNAs or Xeno-miRs) entering circulation through food. The modulating properties of Mediterranean Diet and, more specifically dietary polyphenols, over miRNAs associated to HER2-positive breast cancer has been widely recognized [50]. In this connection, a research conducted by Kwon *et al.* in 2020 on breast cancer survivors assessed the influence of the Mediterranean Diet on plasmatic miRNA signatures using the NanoString human miRNA panel (NanoString, Seattle, WA, USA) [51]. Apart from the observed anthropometric improvements after eight weeks of dietary intervention, the expression of 42 extracellular miRNA was significantly modified. The selected miRNAs were related to breast cancer and energy metabolism, and they might be involved in the cardiometabolic risk of overweight breast cancer survivors. Likewise, a set of miRNAs altered in breast cancer and modulated by diet and exercise was identified by Falzone *et al.* using miRNA

expression microarray datasets available in the GEO DataSets database [52].

Certain natural compounds such as ursolic acid, a pentacyclic triterpene acid widely distributed in plants and waxy shell fruits, can reverse chemotherapy resistance of breast cancer cells by targeting miRNAs [53]. Interestingly, the epigenetic mechanism of action of ginsenoside Rg3 (ginseng) for suppression of breast cancer cell proliferation implicates induction of hypermethylation and inhibition of specific miRNA, combined [36,54].

Understanding food role in breast cancer regulation and metabolism

The so-called post-genomic tools (transcriptomics, proteomics, metabolomics) have undergone a remarkable development in the last decades, offering an amazing opportunity for holistic analysis and genetic and metabolic understanding of diseases. The integration of these large-scale molecular profiling approaches in breast cancer research has enabled the understanding of gene regulation and transcriptional and translational products (proteins and metabolites). More importantly, these technologies can provide additional supportive information in drug development and clinical assessment [55]. The Multi-Omics Breast Cancer Database (MOBCdb), a comprehensive database integrating genomic, transcriptomic, epigenomic, clinical, and drug response data of different subtypes of breast cancer, is noteworthy [56].

Transcriptomics

Transcriptomics can be defined as the assessment of gene expression through the large-scale determination of messenger RNA (mRNA) and/or other classes of RNA molecules such as small interfering RNA (siRNA) or Piwi-interacting RNA (piRNA). Thanks to the high-throughput capabilities of microarrays and RNA-seq, the direct characterization of thousands of transcripts is possible nowadays. Even though non-coding RNAs, including microRNAs (miRNAs), are formally covered by transcriptomics, the importance of these molecules in breast cancer development has been reviewed in the previous section "Food as epigenetic driver in breast cancer", for obvious reasons.

RNA-seq has been used to explore the potential transcriptomic regulators of anacardic acid action in ERα-positive MCF-7 and MDA-MB-231 triple-negative breast cancer cells [57•]. Anacardic acid is a potential dietary agent for preventing and treating breast cancer, initially isolated from cashew nuts. The aforementioned study highlighted differentially regulated miRNAs, mRNA and lncRNA transcripts. The same technology has confirmed an important modification of gene expression upon docosahexaenoic acid (DHA) incorporation in the same triple-negative cell line (MDA-MB-231) [58]. Similarly, gene expression microarrays have been used to

investigate the potential of folic acid for metabolic reprogramming of triple-negative breast cancer in mouse cell models [59].

In a study performed on animal models, Li *et al.* [16] combined tumor observation and histology with epigenetics and transcriptomics. The transcriptome, measured by next-generation mRNA sequencing, revealed significant increase of tumor-suppressor genes transcription and decreased expression of tumor-promoting ones upon prenatal exposure to broccoli sprout diet (sulforaphane). Interestingly, the epigenetic regulatory properties of broccoli were suggested as relevant factors in these transcriptomic findings. In connection to this, an integrated multi-omics (genome-wide DNA methylation analysis, proteomics and metabolomics) data analysis of MCF-7 cells treated with estradiol or/and sulforaphane by Huang *et al.* [37••] highlighted a set of differentially methylated genes and differentially expressed proteins and metabolites, indicating that sulforaphane from broccoli may reverse the adverse effects induced by estradiol.

Proteomics
Proteomics is defined as the study of all proteins enclosed in a specific biological matrix at a given point in time, *i.e.* the proteome. This discipline has evolved in parallel to mass spectrometry developments, in particular, electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) techniques. A quantitative proteomics approach reported by Roehrer *et al.* in 2019 [60] provided a comprehensive view on the proteome composition of MCF-7 cells treated with a minor hop compound, xanthohumol C. Differences in protein expression were detected, providing insights in molecular mechanisms and possible target structures of this bioactive compound. Proteomics has been also used to determine the changes induced by citrus flavonoid 2′-hydroxyflavanone in the proteins responsible for breast cancer incidence, metastases and therapeutic sensitivity [61]. In a similar fashion, the proteomics profiling of MCF-7 estrogen responsive and MDA-MB-231 estrogen non-responsive adenocarcinoma cell lines, exposed to different concentrations of genistein, daidzein and soy seed extracts, revealed that isoflavones affected distinct molecular pathways in both types of cancers such as tyrosine kinases signaling pathway, cytoskeleton organization, lipid and phospholipid catabolism, extracellular matrix degradation and mRNA splicing [62]. In addition, those changes were dose-dependent and affected distinctly to ER-positive and ER-negative cells.

Metabolomics
Metabolomics is defined as the study of all small molecules (metabolites) enclosed in a specific biological matrix at a given point in time, *i.e.* the metabolome. As the end product of genome, the metabolome is a complex constituting amalgamation of widely diverse primary and secondary metabolites that can be characterized in a targeted or an untargeted manner using different techniques. This omics discipline relies mainly in nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) developments, in combination to different separation alternatives such as liquid and gas chromatography. In 2020, a metabolomics exploratory study by Lécuyer *et al.* [64] with 200 cases and matched controls was performed by untargeted LC-MS in order to select diet-related metabolites discriminating women at higher risk of breast cancer. Almost six hundred metabolites were selected in plasma from women who subsequently developed breast cancer, including a pepper compound, a plasticizer, a steroid sulfate and a metabolite linked to microbiota. Also metabolomics has permitted to stablish an association between BMI and increased breast cancer risk in postmenopausal women, underlining four metabolites as biomarkers of carcinogenesis DHEA sulfates [65]. Recent metabolomics studies have related dietary phytochemicals such as polyphenols and coffee methylxanthines with chemopreventive activity in breast cancer [10,66]. These studies have found increased sulfation activity in breast tumor cells, in comparison to normal tissues. Likewise, sulfated metabolites such as 16α-hydroxy DHEA 3-sulfate and pregnene-triol sulfate have been associated with postmenopausal breast tumors and ER-positive neoplasms [64,65]. In agreement with the proteomics findings of Ilieş *et al.* [62], a metabolomics study showed that the phytoestrogen genistein and other dietary estrogens like zearalenone counteract the metabolic and anti-proliferative effect of Palbociclib/Letrozole combined therapy *in vitro* [63]. Interestingly, a volatomics approach was proposed by Silva *et al.* in 2019 [67] to identify potential breast cancer biomarkers. Four volatile metabolites commonly related to diet (limonene, decanoic acid, acetic acid and furfural) presented the highest contribution towards discrimination of breast-cancer and cancer-free tissues. Additionally, this large-scale measurement of volatile organic metabolites proved to be a powerful strategy to complement traditional diagnostics alternatives.

Metagenomics in breast carcinogenesis
The understanding of the role of gastrointestinal and local microbiota in breast carcinogenesis has increased tremendously in the last decade thanks to the emergence of next generation sequencing (NGS) technologies [68,69]. In this context, metagenomics can be defined as the shotgun characterization of total DNA in an organism, in practice, frequently applied to human microbiome (microbial genes harbored by each person) through sequencing of marker genes such as 16S rRNA. The role of human microbiota in breast cancer seems to be mediated by bioactive bacterial metabolites and dysbiosis, but its specific mechanisms remain unresolved [69]. Diet is a major modulator of microbiota, and as such, alcohol, food,

nutrients and/or other food-derived bioactive components, are strong modifiers microbiota.

A Mediterranean study performing 16S rRNA gene-based profiling of the breast tissue microbiota suggested a dysbiosis occurring prior to cancer, establishing a microenvironment prone to cancer [70]. Other authors have pointed out the mediator role of gut microbiota between diet and host's epigenome, more specifically, by producing hormones and/or metabolites that modulate DNA methylation and histone modifications [69,71]. In particular, the 'estrobolome' has been defined as the aggregate of enteric bacterial genes whose products are capable of metabolizing estrogens, that may affect the risk of developing postmenopausal ER-positive breast cancer [72•]. The enzymes involved in conjugation and deconjugation of estrogens are especially important since they impact the host's estrogens metabolism and may influence estrogen-driven neoplasia like breast or endometrial cancer. Already in 2006, Cavalieri *et al.* discussed the role of catechol estrogen quinones as initiators of breast and other human cancers, as well as their implication in monitoring and prevention [73]. These estrogen metabolites can react with DNA to form adducts that may lead to the mutations that initiate cancer, and their levels are a result of imbalanced estrogen-related enzymes. In this context, the gut microbial β-glucuronidases can reactivate estrogens and are considered important components of the estrobolome [74]. Using Illumina MiSeq NGS profiling, a very recent cross-sectional study has associated dietary fiber to gut microbiota with β-glucuronidase activity in postmenopausal breast cancer patients, implying an inverse association between soluble fiber and estradiol levels [75], though more studies are needed in this sense.

An interesting ongoing case-control clinical trial (registration number NCT03885648 in ClinicalTrials.gov) was registered by Plaza-Díaz *et al.* to study the association of breast and gut microbiota dysbiosis and the risk of breast cancer [76]. Metagenomics and metabolomics studies will be carried out in stool and breast tissue samples, along with quantitation of estrogens, estrogen metabolites and endocrine disruptors in serum, urine and breast tissue. The results obtained will contribute for sure to elucidate the role of microbiota in breast health.

Conclusions

As defined by Dr. Alejandro Cifuentes in 2009, *foodomics* is a *discipline that studies the Food and Nutrition domains through the application of omics technologies*. In this context, omics technologies such as nutrigenomics, epigenomics, transcriptomics, proteomics and metabolomics, amongst others, have contributed greatly to the understanding of food role in breast cancer pathogenesis. Food is not and cannot be considered a mere and passive fuel of human health machinery, but instead, it is an active participant.

Also, the symbiotic microbial communities harbored by each person can contribute to human metabolism and disease development.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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