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REVIEWS: CURRENT TOPICS

Plant flavonoids in cancer chemoprevention: role in genome stability

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Abstract

Carcinogenesis is a multistage process that involves a series of events comprising of genetic and epigenetic changes leading to the initiation, promotion and progression of cancer. Chemoprevention is referred to as the use of nontoxic natural compounds, synthetic chemicals or their combinations to intervene in multistage carcinogenesis. Chemoprevention through diet modification, *i.e.*, increased consumption of plant-based food, has emerged as a most promising and potentially cost-effective approach to reducing the risk of cancer. Flavonoids are naturally occurring polyphenols that are ubiquitous in plant-based food such as fruits, vegetables and teas as well as in most medicinal plants. Over 10,000 flavonoids have been characterized over the last few decades. Flavonoids comprise of several subclasses including flavonols, flavan-3-ols, anthocyanins, flavanones, flavones, isoflavones and proanthocyanidins. This review describes the most efficacious plant flavonoids, including luteolin, epigallocatechin gallate, quercetin, apigenin and chrysin; their hormetic effects; and the molecular basis of how these flavonoids contribute to the chemoprevention with a focus on protection against DNA damage caused by various carcinogenic factors. The present knowledge on the role of flavonoids in chemoprevention can be used in developing effective dietary strategies and natural health products targeted for cancer chemoprevention.

Keywords: Cancer; Flavonoids; Chemoprevention; Dietary antioxidants; DNA damage signaling and repair; Polyphenols; DNA damage detection; Genome stability

1. Introduction

The term *cancer* can be described as a set of complex processes involving impaired cells death, unlimited cell proliferation and temporal–spatial changes in cell physiology that often leads to malignant tumor formation resulting in invasion of distant tissues to form metastasis [1]. Multistage carcinogenesis is a widely accepted hypothesis in the development of cancers and is operationally divided into three stages, namely, initiation, promotion and progression [2,3]. Carcinogenesis may result from extensive DNA damage, often caused by exposure to a variety of exogenous and endogenous agents including ultraviolet radiation (UVR), ionizing radiations (IRs), mutagenic chemicals, environmental agents, therapeutic agents or diagnostic imaging. *DNA damage* as a term encapsulates both frank single and double-stranded DNA breaks, as well as stable modifications to nitrogen bases in DNA or its sugar-phosphate backbone, caused by external (*e.g.*, IR) or internal sources [*e.g.*, reactive oxygen species (ROS) generated during oxidative metabolism], which impact the cell by disrupting gene function and/or impairing transcription, DNA replication and cell proliferation [4]. Maintaining genomic integrity is therefore crucial for the organism since it is a key feature in the maintenance of cell function and inappropriate DNA repair is associated with both the initiation and progression of cancer [5].

Failure in proper DNA protection and DNA repair mechanisms, decrease in cellular defenses, malfunctions in cell cycle checkpoints and aberrant inflammatory signaling can contribute to poor genomic stability and provide an "Achilles heel" exploited by many cancer therapeutics [6]. As such, the differences in the DNA damage response between normal and cancer cells often underlie the utility of DNA damaging agents in cancer treatment [7]. DNA damage occurring during "S" phase of cell cycle, when DNA is replicated, was considered as the most lethal DNA damage [8] and, given the uncontrolled proliferation of cancer cells, may explain why DNA-damaging agents can be so effective in targeting cancers.

Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ATM-Rad3-related; BRCA, breast cancer proteins; CHK, checkpoint kinases; DDR, DNA damage response; DNA-PK, DNA-protein kinases; DRI, dose reduction index; DSBs, double strand breaks; EGCG, epigallocatechin-3-gallate; H_2O_2, hydrogen peroxide; HDAC, histone deacetylase; HR, homologous recombination; IF, immunofluorescence; IR, ionizing radiation; MDC1, mediators of DNA damage checkpoint 1; mTOR, the mechanistic target of rapamycin; NHEJ, nonhomologous end joining; PI, propidium iodide; PI3K, phosphatidylinositol-3 kinases; RAD51/52, radiation-induced assembly; ROS, reactive oxygen species; SSBs, single-strand breaks; UVR, ultraviolet radiation.

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2. Cellular DNA-damaging agents

Cellular DNA can be damaged by cytotoxic or genotoxic agents with different mechanism of action. Some of the classical groups of DNA damage inducers used in chemotherapy are alkylating agents, platinum drugs, antimetabolites, topoisomerase inhibitors and forms of ionizing radiation [9]. Alkylating agents like the nitrogen mustards, nitrosoureas, aziridine compounds, alkyl sulphonates and triazine compounds are electrophiles that covalently transfer alkyl groups onto the DNA bases, disrupting the DNA helix shape [10]. Cisplatin and its analogs, carboplatin and oxaliplatin, are DNA-intrastrand cross-linking agents, and these platinum-based drugs are often used to treat testicular or ovarian cancers [11]. The fluoropyrimidine 5-fluorouracil, an antimetabolite having similar structures that are related to nucleotide metabolites, induces DNA damage by either inhibiting biosynthetic processes or being incorporated into nucleic acids such as DNA and RNA [12]. Camptothecin, a plant alkaloid, is a topoisomerase inhibitor widely used to treat colorectal, ovarian and lung cancers that targets DNA-TOP1 (type 1 topoisomerase) cleavage complexes, blocking religation and resulting in the accumulation of transient single-strand breaks (SSBs) [13]. A summary of factors which causes DNA damage is depicted in Fig. 1.

3. DNA damage response (DDR)

The DDR is a complex cascade of molecular and cellular events that is necessary to eliminate lethal and tumorigenic mutations caused by different genotoxic stress including carcinogens produced by physical or chemical sources. This signaling mechanism regulates cell proliferation, cell cycle and apoptotic induction [14]. Failure in proper DNA damage response mechanisms may result in improper DNA repair which can drive to tumorigenesis and can affect sensitivity to genotoxic chemotherapy [10]. DNA repair can be initiated by various enzymes that modify the DNA and nuclear damage by activating polymerases, topoisomerases, ligases, kinases, phosphatases and glycosylases [15]. DDR is commonly activated in early neoplastic lesions and likely protects against malignancy [16].

4. Signal transduction mechanisms in DNA damage

DNA double-strand breaks (DSBs) are well regarded as the most lethal lesions among all types of damages and possess the greatest challenge to human beings. DSBs can be caused by UV, radiotherapy, dysfunctional telomeres or genotoxic agents, and the breaks they induce can activate phosphatidylinositol-3 kinases (PI3K) including ataxia telangiectasia mutated (ATM), ATM-Rad3-related (ATR) or DNA-dependent protein kinases (DNA-PK) that serve as the pinnacle step in DNA damage signaling (Fig. 2). DNA-PK is a multicomponent complex consisting of the DNA-PK catalytic subunit (DNA-PKcs) and the Lupus Ku autoantigen protein heterodimer (Ku80 and Ku70) [17]. Although there is some cross talk in downstream targets of ATM and ATR, these kinases are activated by ionizing irradiation or ultraviolet light/hydroxyurea, respectively [18]. While DNA-PK regulates a small group of proteins involved in DNA DSB end joining, it also cooperates with ATR and ATM to phosphorylate proteins involved in the DNA damage checkpoints [19]. Thus, protein phosphorylation plays a crucial role in DNA damage signaling, activating over 700 proteins in response to DNA damage which in turn counteract genotoxic stresses by regulating protein–protein interactions and other posttranslational modifications [20]. However, the functional significance of many of these proteins is unclear and remains an area of intense research.

DNA is packaged within chromatin, and the reversible acetylation of proteins within chromatin-like histone H3 and H4 can affect DNA repair by a number of mechanisms including altering the association of DNA repair factors with damaged DNA and the modulation of the relaxation (or condensation) state of chromatin that may be important in regulating physical access of the DNA repair machinery to the break within chromatin [7,21]. Acetylation is mediated by histone acetyltransferases (or HATs), and deacetylation is mediated by histone deacetylases (HDACs); the balance of HAT and HDAC activities is often perturbed in cancers. Thus, histone acetylation could serve as a therapeutic target for cancer treatment [22]. For example, HDAC inhibitors have the potential to interfere with DNA repair and chromatin relaxation mechanisms, potentially sensitizing cancer cells to DNA damage [23]. Another critical posttranslational modification of chromatin is phosphorylation, and one of the earliest events during DNA DSB repair is the phosphorylation of Ser139 on the specialized histone H2AX called, which is then referred to as γ-H2AX. H2AX is one of the heteromorphous variants of family of at least eight protein species of the nucleosome core histone H2A [24]. Cytologically, γ-H2AX forms punctate structures in the nucleus known as DNA *repair foci*, which result from the spread of γ-H2AX along chromatin surrounding the DNA break up to 1–2 megabases of DNA *via* the action of ATM. These repair foci serve as platforms for the assembly and recruitment of other DNA repair factors, including mediators of DNA damage checkpoint 1 (MDC1) to initiate the DNA damage response [25].

Once initiated, the DNA damage signal is amplified by checkpoint kinases 1 and 2 (CHK1 and CHK2) that are activated by phosphorylation by ATM and ATR, and a number of effector proteins, including breast cancer antigens 1 and 2 (BRCA1, BRCA2), p53 and murine double minute [26]. Chk1 and Chk2 can phosphorylate DNA repair proteins like BRCA2 and are involved in cell cycle checkpoint control by phosphorylating proteins such as p53 [27]. BRCA1 and BRCA2 are involved in recruiting the repair protein RAD51 to sites of DNA damage to facilitate DSB repair by homologous recombination (HR) [28–30]. The p53 is phosphorylated by Chk1 and Chk2 in response to DNA damage and in turn regulates cell fate decisions including cell cycle arrest and apoptosis by inducing expression of protein such as p21 and B-cell CLL/lymphoma 2 (BCL2), respectively [7,31]. Highlighting the intimate link between DNA repair and cancer, germline or acquired mutations in several DNA repair and signaling factors including BRCA1, BRCA2, ATM, p53 and CHK2 can contribute to the development of cancers affecting multiple organs including breast and ovary, lungs, pancreas and blood (*i.e.*, leukemias) [32–35]. Other common syndromes associated with increased cancer susceptibility include Seckel syndrome (mutated *ATR*), radiosensitive severe combined immunodeficiency disease and ligase IV (LIG4) syndrome (mutated *LIG4*) [36].

After DNA has been repaired, chromatin must be restored to its original state before damage to allow efficient transcription and replication of DNA. Some of the first steps in chromatin restoration include the dephosphorylation of γ-H2AX by the phosphatases PP4 and PP2A, the proteasomal degradation of MDC1 within repair foci, and deacetylation of H3 or H4 lysines by HDAC [37]. Other proteins like the histone chaperones chromatin assembly factor 1 and antisilencing factor 1 also play a crucial role in restoring chromatin structure and cell cycle progression [38]. Failure of these mechanisms may result in epigenetic alterations and thus cause genomic instabilities and its associated diseases [39].

Epigenetic alterations in DNA, such as methylation, and of chromatin, such as modification of histones by acetylation, methylation, and phosphorylation, are increasingly being recognized for their role in health [36,40]. Most of the diseases involving dysregulation of epigenetics are marked with common characteristics such as developmental defects, immunodeficiency, neurological degeneration and cancer predisposition [17]. DNA methylation of cytosine (5-methylcytosine) is a very common epigenetic mechanism involved in controlling DNA structure, chromosome stability, the mobility of viral DNA-repeated elements (transposons, retrotransposons), gene imprinting and gene expression [41]. In tumor tissues, tumor suppressor