Biomedicine & Pharmacotherapy 168 (2023) 115720

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

The preventive effects of broccoli bioactives against cancer: Evidence from a validated rat glioma model

Nieves Baenas [a], Angélica Vega-García [b], Joaquín Manjarrez-Marmolejo [c], Diego A. Moreno [d,*], Iris A. Feria-Romero [b,**]

[a] Department of Food Technology, Food Science and Nutrition, Faculty of Veterinary Sciences, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Espinardo, 30100 Murcia, Spain
[b] Neurological Diseases Medical Research Unit, National Medical Center "Siglo XXI", IMSS, Av. Cuauhtémoc 330, Col. Doctores, 06720 Mexico City, Mexico
[c] Laboratory of Physiology of the Reticular Formation, National Institute of Neurology and Neurosurgery "Manuel Velasco Suárez", Insurgentes Sur 3877, La Fama, 14269 Mexico City, Mexico
[d] Phytochemistry and Healthy Food Lab (LabFAS), Department of Food Science Technology, Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Campus de Espinardo - 25, 30100 Murcia, Spain

ARTICLE INFO

Keywords:
Broccoli aqueous extracts
Sprouts
Sulforaphane
Brain tumor
C6 rat glioma model

ABSTRACT

The aggressive and incurable diffuse gliomas constitute 80% of malignant brain tumors, and patients succumb to recurrent surgeries and drug resistance. Epidemiological research indicates that substantial consumption of fruits and vegetables diminishes the risk of developing this tumor type. Broccoli consumption has shown beneficial effects in both cancer and neurodegenerative diseases. These effects are partially attributed to the isothiocyanate sulforaphane (SFN), which can regulate the Keap1/Nrf2/ARE signaling pathway, stimulate detoxifying enzymes, and activate cellular antioxidant defense processes. This study employs a C6 rat glioma model to assess the chemoprotective potential of aqueous extracts from broccoli seeds, sprouts, and inflorescences, all rich in SFN, and pure SFN as positive control. The findings reveal that administering a dose of 100 mg/kg of broccoli sprout aqueous extract and 0.1 mg/kg of SFN to animals for 30 days before introducing 1×10^4 cells effectively halts tumor growth and progression. This study underscores the significance of exploring foods abundant in bioactive compounds, such as derivatives of broccoli, for potential preventive integration into daily diets. Using broccoli sprouts as a natural defense against cancer development might seem idealistic, yet this investigation establishes that administering this extract proves to be a valuable approach in designing strategies for glioma prevention. Although the findings stem from a rat glioma model, they offer promising insights for subsequent preclinical and clinical research endeavors.

1. Introduction

Diffuse gliomas, encompassing oligodendrogliomas, astrocytomas, and glioblastomas, represent intracranial tumors originating from the glial lineage. They carry a grim prognosis for patients, as effective strategies to prevent tumor regrowth and progression remain elusive across pediatric and adult cases [1]. Presently, no treatment avenues are available. While no definitive epidemiological factors linked to this tumor type have been identified thus far, factors such as extended life expectancy within the population, consumption of processed foods, environmental pollution, radiation exposure, and viral infections, among others, may contribute to its emergence. Moreover,

Abbreviations: AA, antibiotic/antimycotic solution; BFS, bovine foetal serum; D-MEM FK12, Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; FOXM1, Forkhead Box M1; GSLs, glucosinolates; HPLC-DAD-ESI-MSn, high performance liquid chromatography equipped with diode array detector coupled to mass spectrometer using electro spray ionization; H&E, haematoxylin-eosin; GRA, Glucoraphanin; GSLs, glucosinolates.; HSF, horse fetal serum; ITC, isothiocyanates; SFN, sulforaphane; PAR, photosynthetically active radiation; UHPLC-QqQ-MS/MS, ultra high-performance liquid chromatography coupled with triple quadrupole mass spectrometry; VEGF, vascular endothelial growth factor.

* Correspondence to: Laboratorio de Fitoquímica y Alimentos Saludables (LabFAS), CEBAS, CSIC, Campus Universitario de Espinardo -25, E-30100, Murcia, Spain.
** Correspondence to: Unidad de Investigación Médica en Enfermedades Neurológicas, Centro Médico Nacional "Siglo XXI", Av. Cuautémoc 330, Col. Doctores, Ciudad de México, 06720, México.
E-mail addresses: dmoreno@cebas.csic.es (D.A. Moreno), iris.feria@imss.gob.mx (I.A. Feria-Romero).

https://doi.org/10.1016/j.biopha.2023.115720
Received 31 August 2023; Received in revised form 10 October 2023; Accepted 11 October 2023
Available online 13 October 2023

advancements in imaging technologies have facilitated the early detection of these tumors.

Case-control and cohort studies indicate that the intake of *Brassica* vegetables is associated with a diminished risk of various cancer types, notably lung, colon, stomach, and rectal cancers. Nevertheless, these studies have struggled to distinguish between the specific protective impact of *Brassica* vegetables and that of vegetables in a broader sense. Additionally, these investigations have encountered challenges in mitigating biases originating from individuals participating in the case-control studies [2–5]. The consumption of broccoli has demonstrated significant advantages in safeguarding against chronic ailments, including cardiovascular disease, diabetes, obesity, and specific forms of cancer [6,7]. The commendable therapeutic attributes of broccoli sprouts from its abundant nutrient composition and the presence of bioactive compounds, notably glucosinolates (GSLs) and their hydrolysis byproducts known as isothiocyanates (ITC). These bioactive components are predominantly encountered in vegetables belonging to the *Brassicaceae* family [8,9].

Certain variants exhibit more potent tumor-inhibiting attributes among various isothiocyanates, with sulforaphane (SFN) standing out prominently. SFN is notably abundant in broccoli sprouts. The anticancer effects of SFN are extensive and encompass processes like carcinogen detoxification, indirect augmentation of cellular antioxidant capacity, direct cytostatic impact on tumor cells, suppression of angiogenesis-triggering transcription factors such as HIF-1α and c-Myc, curbing basement membrane integrity via diminished matrix metalloproteinase-2 production, attenuation of endothelial cell proliferation, and deployment of its anti-inflammatory potential [10,11]. Thus, this study examined the preventive impact of aqueous extracts derived from various phenological phases of broccoli (*Brassica oleracea* var. *italica*) alongside its bioactive constituent SFN. The assessment investigated their potential to inhibit tumor growth within a glioma rat model.

2. Materials and methods

2.1. Chemicals and cell culture

Chemicals. All LC–MS grade solvents (methanol, acetonitrile and trifluoroacetic acid) were obtained from J. T. Baker (Phillipsburg, NJ, USA). The standards sinigrin and glucobrassicin were obtained from Phytoplan GmbH (Heidelberg, Germany). The standard sulforaphane was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). The standard chlorogenic acid was obtained from Sigma-Aldrich (Chemie Gmbh, Steinheim, Germany), sinapic acid and quercetin-3-glucoside from Fluka Chemika (Neu-Ulm, Switzerland). All water employed in the extraction and the chromatographic analysis was treated with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Cell culture. Rat cell-line C6 (ATCC, USA), D-MEM FK12 media (ATCC, USA); horse fetal serum (HSF, ATCC, USA); bovine foetal serum (BFS, ATCC, USA); antibiotic/antimycotic solution (AA, GIBCO, USA); trypsin- EDTA 0.05% (ThermoFisher); FOXM1 antibody (K19 clone, SantaCruz biotechnology, USA); VEGF antibody (C20 clone, SantaCruz biotechnology, USA).

2.2. Broccoli (Brassica oleracea var italica) plant material

Seeds. The broccoli seeds (Intersemillas S.A.) untreated, were disinfected with a 0.5% bleach solution for 24 h, then washed with distilled water, a part was frozen with liquid nitrogen for subsequent freeze-drying and grinding to obtain the seed sample. Another part of the seeds was used for sprout germination.

Sprouts. Seeds were spread in trays on a sterile cellulose surface (CN Seeds, U.K.), and placed in a growth chamber under controlled environmental conditions as described in Baenas et al. [12]. After 8 days of germination, a portion of the sprouts was harvested, flash frozen in liquid nitrogen, freeze-dried and ground, and other portion was used for the production of adult plants.

Inflorescences/broccoli heads. The sprouts were placed in hydroponic culture containers (15 liters) with a daily change of ½ Hoagland's nutrient solution. After 24 days, they were transplanted to an experimental farm (latitude 38°06' N, 1°02' W. Santomera, Murcia, Spain), using the same solution for irrigation. The environmental parameters were recorded: air temperature (6 – 12 °C minimum, 18 – 24 °C maximum); relative humidity, 20 −40% (minimum), and 70–100% (maximum); and, photosynthetically active radiation (PAR), averaged 1000 μmol $m^{-2}s^{-1}$ during the first 43 days, 500 μmol $m^{-2}s^{-1}$ from day 44–72, and increased to 1500 μmol m $m^{-2}s^{-1}$ on, day 72. Plants harvested on day 95 were frozen in liquid nitrogen, and then freeze-dried and ground.

2.3. Identification and quantification of bioactive compounds (glucosinolates, phenolic compounds and sulforaphane)

Quantitative analysis of glucosinolates (GSLs) and phenolic compounds was carried out simultaneously by a multipurpose method [13], according to their characteristic retention times, fragmentation patterns (MS-, and MSn) by HPLC-DAD-ESI-MSn, and comparison with authentic standards Freeze-dried samples (100 mg) were extracted with 1.5 mL of MeOH 70% and heated at 70 °C for 30 min with occasional vortexing. The supernatant was collected and after centrifugation at 10.000 g (5 min, 4 °C) was filtered (syringe filters, 0.02 μm Anotop 10® plus, Whatman®, Maidstone, UK) and placed in vials for HPLC-DAD-ESI-MSn analyses. Chromatograms were recorded at 227 nm for GSLs, and at 330 nm for phenolic compounds. Sinigrin and glucobrassicin were used as standards for aliphatic and indolic GSLs quantification, respectively. Chlorogenic acid, sinapic acid and quercetin-3-rutinoside were used as standards for chlorogenic acid derivatives, sinapic acid derivatives and flavonol glycosides, respectively.

SFN was analyzed following its MRM transitions in a ultra-high performance liquid chromatography coupled with to a 6460 tandem mass spectrometer with triple quadrupole technology (UHPLC-QqQ-MS/MS, Agilent Technologies, Waldbron, Germany) according to the method described by Dominguez-Perles, et al. [14]. For sample extraction, freeze-dried samples (50 mg) were hydrolyzed with 1.6 mL Milli-Q water, vortexed for 1 min, and kept at room temperature overnight, according to the protocol of Cramer and Jeffery [15].

2.4. Cellular culturing and selection of cells

The rat cell-line C6 was cultured in culture recipients of 75 cm^2 with D-MEM FK12 media, supplemented with 20% HSF; 10% BFS and 2% AA (supplemented D-MEM FK12). The culture plates were kept in an incubator (Water Jacketed, Nuaire, USA) at stable condition, 5% CO_2 at 35 °C for growth and propagation. The sub-cell-cluster (Sub-C6) was obtained with serial dilutions 1:10 from the starting C6-cells. The last dilution was seeded in a 96 wells-plate/100 μl with supplemented D-MEM FK12. The wells with one cell were selected, then, grew to confluence. After that, sub-clusters were recovered with Trypsin- EDTA 0.05% and spread into 6 well-plates and bottles of 75 cm3. The cluster with highest FOXM1 and VEGF protein expression, was selected. Both proteins were detected by immunohistochemistry technique (data not shown).

2.5. Animal model

Thirty-six male weanling Sprague-Dawley rats, 90–110 g at the start of assays, were used. They were contained in acrylic boxes (50×40×40 cm), maintained under 12:12 h light/dark cycles, with Formulab Diet #5008 food and water ad libitum. Treatments were carried out orally for 30 days using a cannula and fresh extracts dissolved in water, before implantation of tumor cells. The animals were divided into six groups (n

= 6 per group):

- Control SS. Treated with 0.9% saline solution;
- Seed. Treated with aqueous extract of freeze-dried broccoli seeds (100 mg/kg, containing 0.1 mg SFN/kg);
- Sprout. Treated with aqueous extract of freeze-dried broccoli sprouts (100 mg/kg, containing 0063 mg SFN/kg);
- Inflorescence. Treated with aqueous extract of freeze-dried broccoli adult vegetable (100 mg/kg, containing 0.022 mg SFN/kg);
- Control OO. Treated with olive oil;
- SFN 0.1. Treated with sulforaphane oil solution (0.1 mg/kg); and
- SFN 0.7. Treated with sulforaphane oil solution (0.7 mg/kg).

The surgical procedures were done in the vivarium of National Medical Center "Siglo XXI", IMSS by using all the aseptic and antiseptic protocols and materials. The project followed the local laws for animal care NOM-062-ZOO-1999, and the ARRIVE guidelines for avoiding suffering to involved animals. The local ethics committee approved this protocol (Registering Number: R-2015–785–098). Stereotaxic surgical approach was done in anesthetized animals (90 mg/kg ketamine + 10 mg/kg xylazin). Once the head was fixed, the skull was exposed to a trephine was done at anteroposterior 2 mm, lateral 2 mm and deep 2 mm from bregma at this site [16], 1×10^4 cells suspended in D-MEM FK12 medium (ATCC, USA) were injected and the skin was sutured. Then, a 5-day recovery period was permitted, being at the first three days treated with antibiotics (Gentamicin dose + Benzathine penicillin 1200, 000 IU) and analgesic (Tramadol dose, 10 U). The animals were sacrificed three weeks after implantation, due to an overdose of pentobarbital (100 mg/kg), perfused with 4% paraformaldehyde to obtain and dissect the brains for posterior image acquisition, and finally paraffin embedding. Representative slices of 5 μm slices were obtained for tumor identification and haematoxylin-eosin (H&E) staining. The images were photographed with a NI5_Elements D (5.110064 bit, Nikon, Japan) microscope at 40X.

2.6. Image acquisition

Figures were acquired from the perfused and fixed brains by using a VARIAN 7 Tesla Scanner. The sequences were obtained by standard procedure: t1-weighted, sequence Gradient Echo 3D (GE3D), with the acquisition parameters TR 4 ms and TE 2.3 ms, FOV 64 mm × 100 mm, and a matrix of 256 × 256; t2-weighted, sequence Fast Spin Echo Multislice (FSEMS); TR 2952 ms and TE 40 ms, FOV 64 × 64 mm and a matrix of 256 × 256.

2.7. Statistical analysis

Differences in tumor size were analyzed using the Mann-Whitney U nonparametric median comparison statistical test, performed by using the Prima STAT v 12.0 software, a p-value < 0.05 was considered significant.

3. Results

3.1. Chemical composition of the plant material

This study identified ten distinct GSLs within broccoli samples (Table 1). Glucoraphanin (GRA) emerged as the most prevalent compound across all samples, regardless of the broccoli's phenological stage. GRA constituted 50% of total GSLs in seeds, 40.5% in sprouts, and 30% in broccoli heads. Moreover, the GSL profiles in seeds and sprouts exhibited greater similarity when juxtaposed with inflorescence ones. In this context, a higher concentration of glucoiberin and glucoerucin was detected in seeds and sprouts, conspicuously absent in the inflorescence. Concerning the indole-type GSL, 4-hydroxyglucobrassicin emerged as the principal GSL in seeds and sprouts, while in broccoli heads, the predominant ones were glucobrassicin and neoglucobrassicin. Notably, seeds and sprouts exhibited a heightened concentration of aliphatic-type GSLs, while the broccoli heads showcased a more substantial amount of GSLs of the indole group. The findings concerning total GSL content are as follows: seeds (30.2 ± 2.2 mg/g DW), sprouts (32.71 ± 0.25 mg/g DW), and inflorescences (13.47 ± 1.34 mg/g DW) (Table 1). It is worth noting that these results fall within the range of values previously documented by other researchers [8,17,18].

Table 1
Quantification of glucosinolates and phenolic compounds in broccoli plant material.

Compounds	Seed mg/g DW ± SD	Sprout mg/g DW ± SD	Inflorescence mg/g DW ± SD
Glucosinolates			
Glucoiberin	4.42 ± 0.48	4.04 ± 0.12	–
Glucoraphanin	15.22 ± 0.89	13.23 ± 0.00	4.08 ± 0.33
Glucoalysin	–	–	0.19 ± 0.05
Gluconapin	0.40 ± 0.16	–	–
Glucoiberverin	0.72 ± 0.06	–	–
4-Hydroxyglucobrassicin	3.74 ± 0.27	2.96 ± 0.07	0.79 ± 0.07
Glucoerucin	4.63 ± 0.31	7.17 ± 0.09	–
Glucobrassicin	0.40 ± 0.05	1.15 ± 0.01	3.67 ± 0.44
4-Methoxyglucobrassicin	0.24 ± 0.00	1.74 ± 0.02	0.76 ± 0.05
Neoglucobrassicin	0.42 ± 0.02	2.42 ± 0.13	3.97 ± 0.40
ΣAliphatic GSLs	25.39 ± 1.90	24.45 ± 0.03	4.27 ± 0.38
Σ Indol GSLs	4.81 ± 0.34	8.26 ± 0.23	9.19 ± 0.96
Σ Totals	30.20 ± 2.24	32.71 ± 0.25	13.47 ± 1.34
Phenolic compounds			
Chlorogenic acid derivatives		0.40 ± 0.07	0.99 ± 0.21
Flavonol glycosides	0.52 ± 0.03	0.17 ± 0.04	0.49 ± 0.15
Sinapic acid derivatives	12.35 ± 0.56	6.53 ± 1.21	0.73 ± 0.08
Σ Totals	12.87 ± 0.59	7.10 ± 1.32	2.21 ± 0.31

Mean values (n = 3) ± SD (standard deviation), DW (dry weight)

While cruciferous vegetables are renowned for their GSL content, they also harbor a notable concentration of phenolic compounds. Among these, sinapic acid derivatives constitute a significant portion, accounting for approximately 80–98% of the total. Conversely, derivatives of chlorogenic acid and flavonol glycosides exhibit comparatively lower levels. Similar to the GSL trend, the highest concentration of phenolic compounds was evident in the sprouts (27.1 ± 6.3 mg/g DW), succeeded by the seeds (14.2 ± 1.1 mg/g DW), with the broccoli heads exhibiting a subsequent lower content (3.2 ± 0.4 mg/g DW) (Table 1).

Within this study, SFN content was evaluated within the freeze-dried aqueous extracts obtained from the plant samples. Among these samples, the seeds exhibited the most substantial SFN content (1.06 ± 0.06 mg/g DW), trailed by the sprouts (0.63 ± 0.06 mg/g DW) and the inflorescences (0.22 ± 0.05 mg/g DW) (Table 2). This hierarchy aligns with the precursor GSL content, specifically GRA. These outcomes imply that approximately 5% of the total GRA content converts into SFN

Table 2
Quantification of sulforaphane and phenolic compounds in the broccoli aqueous extracts.

Compounds	Seed mg/g DW ± SD	Sprout mg/g DW ± SD	Inflorescence mg/g DW ± SD
Isothiocyanate			
Sulforaphane	1.06 ± 0.06	0.63 ± 0.06	0.22 ± 0.05
Phenolic compounds			
Chlorogenic acid derivatives	0.002 ± 0.00	0.004 ± 0.00	0.24 ± 0.01
Flavonols	0.008 ± 0.00	0.002 ± 0.00	0.05 ± 0.00
Sinapic acid derivatives	0.20 ± 0.03	0.03 ± 0.00	0.24 ± 0.01
Σ Totals	0.21 ± 0.03	0.04 ± 0.004	0.54 ± 0.01

Mean values (n = 3) ± SD (standard deviation), DW (dry weight)

following the maceration of the freeze-dried samples in water and the enzymatic activity of myrosinase present within the samples. This conversion is influenced by an array of factors, encompassing plant species and various pre- and post-harvest elements that impact the presence of specific epithiospecifier proteins [19].

Moreover, congruent with the outcomes pertaining to the initial plant material, sinapic acid derivatives emerged as the primary category of phenolic compounds. Notably, higher concentrations of these derivatives were observed in the inflorescences (0.54 ± 0.01 mg/g DW), succeeded by the seeds (0.21 ± 0.03 mg/g DW), and lastly, the broccoli sprouts (0.04 ± 0.00 mg/g DW) (Table 2).

3.2. Effect of aqueous broccoli extracts in the prevention of tumor development

In these experiments, an experimental model was formulated to observe the potential antineoplastic effects of broccoli. Accordingly, various aqueous extracts from three plant phenological stages (seeds, sprouts, and inflorescence) were administered to rats over 30 days before the introduction of C6-glioma cells. This specific time frame corresponds to a critical risk factor and the initiation of the tumor niche in humans. The objective was to assess whether this consumption could protect against tumor development. Fig. 1 illustrates the intracranial astrocytic tumor alongside its corresponding histological sections. The control SS and seed groups exhibited substantial tumor growth, accompanied by histopathological traits indicative of high-grade gliomas. These traits encompassed cell proliferation, thrombosed vessels exhibiting endothelial hyperplasia, and areas of necrosis. Within the inflorescence group, approximately half of the subjects manifested tumors with a volume of $\leq$ 15 mm^3. Despite this size reduction, histopathological analysis continued to reveal characteristics consistent with high-grade astrocytomas. Conversely, the sprout group displayed no discernible evidence of macroscopic tumors. However, diffuse low-grade tumors characterized by cellular proliferation and parenchymal infiltration were detected during the histological examination.

The tumor volume within the control SS group significantly increased compared to the sprout group (39.43 mm^3 ± 12.93 vs 8.08 mm^3 ± 2.45, p = 0.0022) (Fig. 2). However, the volume fluctuations observed in the seed and inflorescence groups did not demonstrate any statistically significant differences when compared to the control group (39.43 mm^3 ± 12.93 vs. 94.37 mm^3 ± 65.94, p = 0.2831; 39.43 mm^3 ± 12.93 vs. 32.20 mm^3 ± 34.34, p = 0.3430, respectively).

3.3. Effect of pure SFN on the prevention of tumor development

In these experimental groups, akin to the employment of broccoli aqueous extracts, the primary objective was to ascertain whether SFN can hinder tumor growth when administered prior to the implantation of tumor cells. Fig. 3 illustrates the process of tumor development within the control OO group and two varying concentrations of SFN (0.1 and 0.7 mg/kg) alongside their corresponding H&E-stained sections. In this experiment, olive oil was chosen as the vehicle due to SFN's low polarity, enabling better dissolution and preservation in this solvent than other polar solvents. Initially, it was noted that the Control OO group did not exhibit substantial tumor growth compared to the saline control group. Nevertheless, the histopathological characteristics indicative of a diffuse high-grade glioma were present.

Similarly, within the SFN 0.7 mg/kg group, a propensity for high-grade glioma development was evident, exhibiting a significantly larger size compared to the Control OO group (12.24 mm^3 ± 7.03 vs. 60.54 mm^3 ± 58.59, p = 0.0087). In contrast, the SFN 0.1 mg/kg group significantly reduced tumor size compared to the Control OO group (12.24 mm^3 ± 7.03 vs. 4.44 mm^3 ± 3.35, p = 0.0152). This latter group also displayed histopathological attributes characteristic of a diffuse low-grade glioma (Fig. 4).

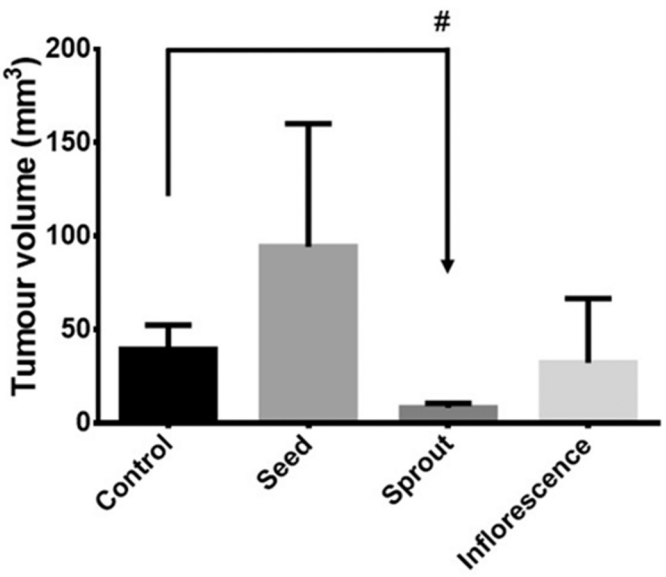

Fig. 2. Effect of treatment with different aqueous extracts of broccoli on tumor volume. After 3 weeks of implantation with 1 × 10^4 C6-cells, the tumor volume of rats was previously treated with saline solution (Control) or 100 mg/kg of aqueous extract of seeds, sprouts or inflorescences. The Mann-Whitney test determined significant differences. # = p < 0.005.

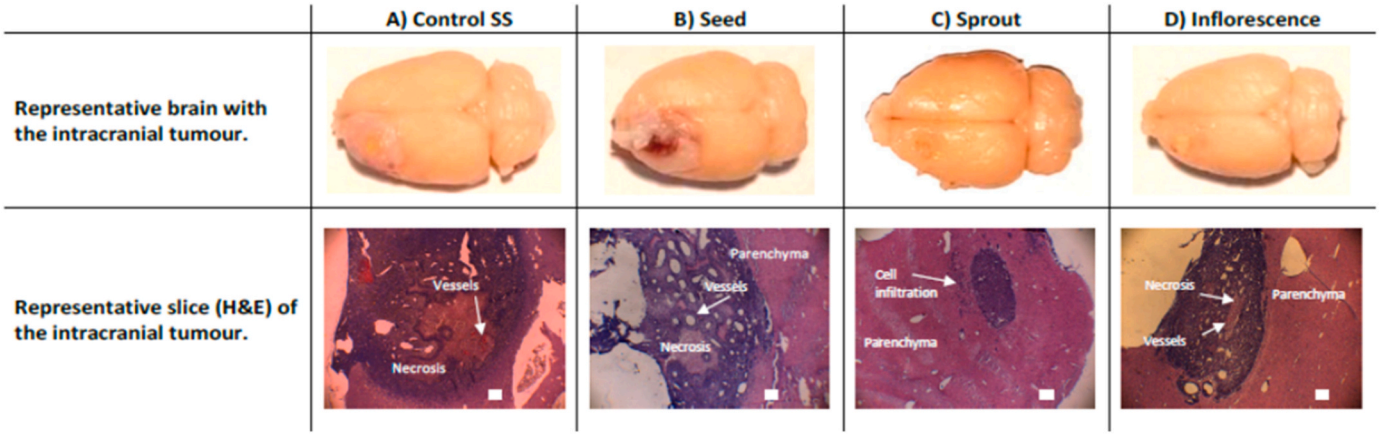

Fig. 1. Brain slices stained with H&E from rats implanted with 1 × 10^4 C6-cells and treated with different aqueous broccoli extracts. Tumor volume of 3 weeks post-implant previously treated with saline solution (A) or 100 mg/kg of aqueous extract of seeds (B), sprouts (C) and inflorescences (D). Scale bar 120 μm.

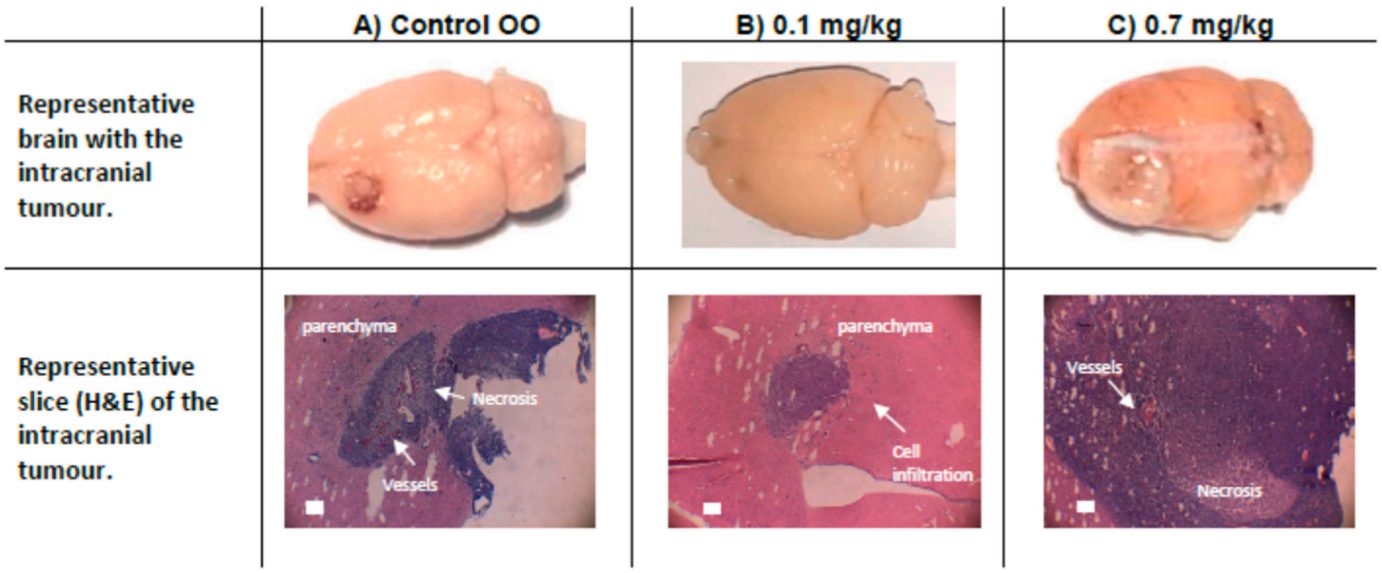

Fig. 3. Brain and slices stained with H&E from rats implanted with 1×10^4 C6-cells and treated with different concentrations of SFN. Tumor volume of 3 weeks post-implant previously treated with Olive oil (A), 0.1 mg/kg of SFN (B) or 0.7 mg/kg of SFN (C). Scale bar 120 μm.

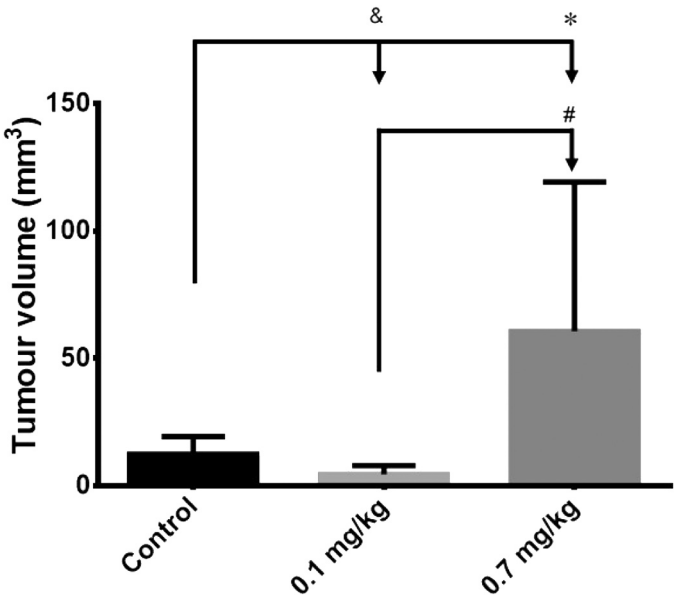

Fig. 4. Effect of treatment with different concentrations of SFN on tumor volume. After 3 weeks of implantation with 1×10^4 C6-cells, the tumor volume of rats was previously treated with Olive oil (Control), 0.1 mg/kg or 0.7 mg/kg of SFN. The Mann-Whitney test determined significant differences. # = $p < 0.005$, *= $p < 0.01$ and & = $p < 0.05$.

4. Discussion

To this day, the exact risk factors associated with the development of human gliomas remain uncertain. However, there is a consensus on the multifactorial alterations that can contribute to the formation of glioma cells. These include age-related changes in the origin cells, the brain microenvironment, the accumulation of mutations in neural stem cells leading to their transformation into glioma-forming cells, and diminished immune surveillance [20,21]. Concerning this, a recent systematic review and meta-analysis encompassing 7278 cases of glioma revealed that a high consumption of vegetables significantly reduces the risk of gliomas (RR = 0.84; 95% CI: 0.73–0.96; P = 0.012) compared to a low intake [22]. This underscores the importance of having substantial evidence regarding the preventive effects of vegetable consumption, including vegetables like broccoli, against glioma formation within an experimental model.

The current study investigates the chemopreventive properties of the aqueous extract obtained from three physiological stages of broccoli: seeds, sprouts (seedlings), and inflorescences (commonly referred to as adult vegetables or broccoli heads), concerning their impact on glioma growth and progression.

In the rat glioma transplantation model, Auer et al. demonstrated that intracranial injection of 104 glioma-C6 cells led to the development of astrocytoma exhibiting malignant features, such as diffuse infiltrating edges and necrosis, in 100% of the subjects [23]. This indicates that even this minimal quantity of cells creates a conducive brain environment for tumor growth and progression. In this study, all experimental groups developed gliomas; however, the group administered with the aqueous extract of broccoli sprouts and the group treated with pure SFN at 0.1 mg/kg exhibited the formation of diffuse low-grade gliomas. Conversely, the remaining groups exhibited the malignant characteristics documented by Auer et al. and manifested the development of thrombosed vessels. This result suggest that 0.1 mg/kg pure SFN, as the combination of antioxidant compounds included in the aqueous extract of broccoli sprouts at 0.06 mg/Kg exerted a protecting effect for the development of a glioma of high degree. On the other hand, using 0.7 mg/kg of pure SFN, and a natural matrix of compounds as the seeds water extract, did not allow to observe such protective effect.

At the metabolic level, the primary distinction between normal and tumor cells lies in energy generation via glucose degradation, with tumor cells exhibiting greater consumption of this monosaccharide. Glycolysis, the metabolic pathway for glucose leading to ATP generation, occurs in the cell cytoplasm and is a common feature of normal and cancer cells. However, their metabolic outcomes from this pathway differ significantly [24].

In normal cell glycolysis, glucose is broken down into pyruvate and ATP by hexokinase I (HK I). Subsequently, pyruvate enters the mitochondria, transforming into acetyl-CoA through the Krebs cycle. This process generates NADH and FADH2, high-reducing-power molecules, along with ATP. In cancer cells, anaerobic glycolysis (known as the Warburg effect) occurs. Hexokinase II (HK II) breaks down glucose into pyruvate, producing two ATP molecules and various glycolytic intermediates that feed into multiple biosynthetic pathways. However, instead of being oxidized in the mitochondria, pyruvate is converted to

lactate in the cytoplasm through the action of lactate dehydrogenase (LDH). This lactate production acidifies the microenvironment and promotes reactive species production [25].

In the brain, there is evidence of acute aerobic glycolysis (referred to as the Crabtree effect) in astrocytes, triggered by potassium (K^+) and mediated by nitric oxide (NO). This glycolysis occurs alongside slower, more prolonged aerobic glycolysis (Warburg effect) induced by glutamate, possibly in combination with ammonium (NH^{4+}). This combination enables neurons to consume glucose and lactate, maintaining a delicate equilibrium between glycolysis and respiration driven by the sodium pump. Consequently, the Warburg and Crabtree effects do not signify inefficient energy production but rather the orchestrated strategy of astrocytes to provide neuronal ATP [26]. The ensuing oxidative stress facilitates the demise of healthy cells, a process regulated by mitochondria-specific autophagy (mitophagy). Subsequently, an imbalance in mitochondrial fusion-fission activity is initiated (via Ca^{2+}-dependent opening of the permeability transition pore in the inner mitochondrial membrane). In tumor cells, the presence of free radicals sustains mitochondrial dysfunction, fostering the development, progression, and evasion of apoptosis in these cells, along with facilitating metastasis [27].

Brassica vegetables, with prominent broccoli, are recognized for their elevated content of bioactive compounds. Notably, glucosinolates (GSLs) and their hydrolysis products, isothiocyanates (ITC), alongside (poly)phenols (mainly chlorogenic and sinapic acids derivatives, as well as flavonoids), are abundant in these vegetables. In conjunction with other nutrients, these compounds may collaboratively scavenge free radicals and adjust the redox equilibrium [28]. Consequently, they are believed to contribute synergistically to preventing cancer development and progression via various cellular mechanisms.

The concentration of bioactive compounds within broccoli displays variation due to distinct genetic factors (such as varieties or cultivars), environmental influences (encompassing abiotic or biotic stresses), and the vegetable's diverse stages of development [18,29]. Among the plant's reserve organs, the seeds hold a greater abundance of nutrients, including lipids and carbohydrates, but fewer minerals and amino acids than the sprouts [30]. The aqueous extract of the plant matrix (seeds) is used to counteract toxic effects, such as those of erucic acid found in lipids [31]. As the plant matures, the seed's content of nutrients and bioactive compounds significantly diminishes. Notably, 8-day-old sprouts are a substantial source of health-enhancing bioactive compounds. These encompass nitrogen and sulphur derivatives (GSLs), phenolic compounds, minerals (selenium, potassium, and manganese), and vitamins (A, C, K, and B6), with concentrations notably exceeding those in inflorescences [30]. Notably, seeds and sprouts can contain 2–10 times more GSLs than the mature head. In this study, each extract exhibited distinct levels of GSLs, primarily GRA, along with varying degrees of its hydrolysis product, SFN. These GSLs are enzymatically broken down into their corresponding ITCs when plant tissue is disrupted, either through chewing (via the enzyme myrosinase) or post-digestion by microbiota within the digestive tract [32].

One of the most recognized mechanisms through which the ITC SFN contributes to protection against cancer development involves the activation of genes linked to the oxidative stress response. This is achieved by activating the nuclear transcription factor Nrf2, subsequently inducing phase II detoxification enzymes while inhibiting phase I enzymes [11,33]. SFN also safeguards the mitochondrial membrane from oxidative stress's detrimental effects by engaging the Keap1/Nrf2/ARE pathway [34]. A shared characteristic of most Nrf2 activators (including ITCs and phenolic compounds) is their ability to interact with sulfhydryl groups, which leads to oxidation-reduction, alkylation, or disulfide exchange, ultimately chemically altering Keap1 cysteine residues and disrupting the Keap1/Nrf2 complex [35]. Under conditions of oxidative stress, Nrf2 transcriptionally upregulates enzymes with detoxifying and antioxidant capabilities, such as NAD(P)H-quinone oxidoreductase (NQO1), heme oxygenase-1 (HO-1), glutathione S-transferase (GST), thioredoxin reductase (TrxRs), and gamma-glutamylcysteine synthetase (gamma-GCS), in order to mitigate damage induced by free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) [36]. Notably, SFN has been reported to reduce brain inflammation following consumption, with evidence from a rat model of traumatic brain injury suggesting its potential to cross the blood-brain barrier [37]. SFN intake inhibits microglial activation and inflammation by modulating the mitogen-activated protein kinase (MAPK) pathway, particularly from broccoli sprouts. This regulatory effect fosters an unfavorable microenvironment for glioma development, mirroring its impact on neurodegenerative diseases [38].

In this study, the protective effect against the development of a glioma with different concentrations of SFN either in a natural source (with other nutrients in a food matrix), or in pure form was studied. The observed result when using extract of inflorescences or mature broccoli (0.022 mg SFN/Kg) was not enough to exert a protective effect. On the other hand, in the seeds extract (0.1 mg SFN/Kg), other compounds present in the matrix (e.g. antinutrients such as erucic acid, carbohydrates, etc.) inhibited such protective effect of SFN. On the contrary, a water extract of broccoli sprouts with high content of SFN (0.7 mg SFN/Kg), could be in an excessive almost toxic range, inducing natural defense mechanisms in the organism. We can speculate these effects that merit further investigation. Furthermore, it was previously reported that the exposure to SFN, either in vitro or in vivo, led to the overexpression of pivotal drug-efflux pumps within the blood-brain barrier: P-glycoprotein (P-gp), multidrug resistance-associated protein-2 (Mrp2), and breast cancer resistance protein (Bcrp) [39]. These ATP-driven pumps serve as integral components in the blood-brain barrier's defensive and detoxification mechanisms by controlling the entry of xenobiotics into the brain parenchyma. However, these drug-efflux proteins become linked to drug resistance while operating under therapeutic circumstances [40]. In this study, notable tumor growth was observed in both the seed and 0.7 mg/kg SFN treated groups, to the heightened expression of these drug-efflux proteins, could be at least in part, responsible of this observed response. It is plausible to consider that the elevated concentration of bioactive compounds and nutrients in the case of the seeds, and the high level or dosage of pure SFN hindered the entry of SFN into the brain. As a result, the anticipated antioxidant and protective effects against the presence of procarcinogens might not manifest [41,42]. Additionally, the seeds' constituents, such as lipids and carbohydrates, could render this aqueous extract less efficacious in this model. This reduced efficacy could also be attributed to certain antinutrients (such as erucic acid) that might not contribute positively to the desired biological outcome. Broccoli sprouts exhibited a remarkable GSL content, primarily GRA, yielding an aqueous extract containing 0.63 mg/g DW of SFN. This compound assumes a pivotal biological role in this study, demonstrating enhancements across all pathophysiological parameters associated with the disease. Human consumption of SFN has exhibited notable bioavailability, ranging between 70% and 90% [6,43]. Moreover, broccoli sprouts encompass supplementary phenolic compounds beyond ITCs, and potentially additional vitamins and nutrients. This assortment of bioactive elements could induce a favorable food "matrix effect".

Subjects administered with 0.1 mg/kg of SFN displayed a notable decrease in tumor growth; a stark contrast is evident when compared to the corresponding control group (control OO) and the 0.7 mg/kg SFN-treated group. These findings substantiate the notion that elevated doses of the pure compound might potentially assume a pro-oxidant role, while lower doses hold promise in fostering an environment rich in antioxidants and conducive to chemoprotection [6,44].

The outcome from the adult head or inflorescence group, which possesses a lower GSLs content compared to the sprouts due to their advanced physiological state, exhibited less significant biological activity than the group treated with the sprouts, though it was still superior to the group treated with the seeds.

In the context of preventing chronic diseases through the adjunctive

impact of natural foods in the diet, incorporating broccoli sprouts into one's regular diet emerges as a highly promising strategy for averting the onset of specific types of cancer. Moreover, it is the most cost-effective option for society when contrasted with the consumption of nutraceuticals. While achieving a standardized effective dose of SFN via food consumption, such as sprouts, poses challenges, forthcoming clinical studies are poised to determine an appropriate dosage for shaping human intervention trials. These trials would employ broccoli sprouts as a foundational component in the dietary matrix aimed at preventing cancer and various other ailments.

5. Conclusions

This preclinical study demonstrates the preventive effect of broccoli consumption on brain cancer development using a rat glioma model that develops a malignant tumour due to the number of implanted cells in untreated healthy rats. Among the different broccoli phenological stages and SFN doses tested, broccoli sprouts and SFN 0.1 mg/kg showed significant inhibition of tumour growth and implant progression. This work demonstrates the importance of searching for foods and/or formulations rich in bioactive compounds of natural origin (e.g. broccoli and its derivatives) that can be used as preventive treatments in the design of strategies and programs for the prevention of chronic and degenerative diseases such as cancer.

Funding

This work was supported by the Health Research Coordination-IMSS through financing FIS/IMSS/PROT/G17-2/1725. NB is granted by a "Juan de la Cierva Incorporación"(IJC-2020-044496-I) post-doctoral contract funded by the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033). This research was partially supported by Fundación Seneca - Murcia Regional Agency for Science and Technology (CARM), Project Reference N# 20855/PI/18.

CRediT authorship contribution statement

Nieves Baenas: Investigation, Methodology, Resources, Writing – original draft. **Angélica Vega-García**: Methodology Resources. **Joaquín Manjarrez-Marmolejo**: Investigation, Methodology, Validation. **Diego A. Moreno**: Conceptualization, Investigation, Resources, Visualization and Writing. **Iris Angélica Feria-Romero**: Conceptualization, Data curation, Investigation, Funding acquisition, Resources, Validation, Visualization and Writing.

Declaration of Competing Interest

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.

Data availability

All data generated or analyzed during this study are included in this published article. Enquiries about data availability should be directed to the authors.

Acknowledgements

We thank Mr. Francisco Guerra O'Hart for his financial support to the social service student Ricardo Bravo who was in charge of the animals. We thank the staff of the vivarium M en C Itzel Isaura Vaca Ibarra, MVZ Julio García Hernández and Nurse Fabiola Ortíz Pérez by their support in the care and surgery of the animals.

References

[1] N.A. Mohile, H. Messersmith, N.T. Gatson, A.F. Hottinger, A. Lassman, J. Morton, D. Ney, P.L. Nghiemphu, A. Olar, J. Olson, J. Perry, J. Portnow, D. Schiff, A. Shannon, H.A. Shih, R. Strowd, M. van den Bent, M. Ziu, J. Blakeley, Therapy for diffuse astrocytic and oligodendroglial tumors in adults: ASCO-SNO guideline, J. Clin. Oncol. 40 (2022) 403–426, https://doi.org/10.1200/JCO.21.02036.
[2] G. van Poppel, D.T.H. Verhoeven, H. Verhagen, R.A. Goldbohm, Brassica vegetables and cancer prevention. Epidemiology and mechanisms, Adv. Exp. Med. Biol. 472 (1999) 159–168, https://doi.org/10.1007/978-1-4757-3230-6_14.
[3] J. Bielecka, R. Markiewicz-żukowska, The influence of nutritional and lifestyle factors on glioma incidence, Nutrients 12 (2020) 1–20, https://doi.org/10.3390/NU12061812.
[4] C. Conaway, Y. Yang, F. Chung, Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans, Curr. Drug Metab. 3 (2005) 233–255, https://doi.org/10.2174/1389200023337496.
[5] M.T. López-Chillón, C. Carazo-Díaz, D. Prieto-Merino, P. Zafrilla, D.A. Moreno, D. Villaño, Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects, Clin. Nutr. 38 (2019) 745–752, https://doi.org/10.1016/j.clnu.2018.03.006.
[6] Y. Yagishita, J.W. Fahey, A.T. Dinkova-Kostova, T.W. Kensler, Broccoli or sulforaphane: is it the source or dose that matters, Molecules 24 (2019) 3593, https://doi.org/10.3390/molecules24193593.
[7] Y. Zhang, Cancer-preventive isothiocyanates: measurement of human exposure and mechanism of action, Mutat. Res. Fundam. Mol. Mech. Mutagen. 555 (2004) 173–190, https://doi.org/10.1016/j.mrfmmm.2004.04.017.
[8] H. Lin, J. Sun, Z. Hu, C. Cheng, S. Lin, H. Zou, X. Yan, Variation in glucosinolate accumulation among different sprout and seedling stages of broccoli (Brassica oleracea var. italica), Plants 11 (2022), https://doi.org/10.3390/plants11121563.
[9] A.P. Vale, J. Santos, N.V. Brito, D. Fernandes, E. Rosa, M. Beatriz, P.P. Oliveira, Evaluating the impact of sprouting conditions on the glucosinolate content of Brassica oleracea sprouts, Phytochemistry 115 (2015) 252–260, https://doi.org/10.1016/j.phytochem.2015.02.004.
[10] B. Licznerska, H. Szaefer, V. Krajka-Kuźniak, R-sulforaphane modulates the expression profile of AhR, ERα, Nrf2, NQO1, and GSTP in human breast cell lines, Mol. Cell. Biochem. 476 (2021) 525–533, https://doi.org/10.1007/s11010-020-03913-5.
[11] Q. Wang, Y. Bao, Nanodelivery of natural isothiocyanates as a cancer therapeutic, Free Radic. Biol. Med. 167 (2021) 125–140, https://doi.org/10.1016/j.freeradbiomed.2021.02.044.
[12] N. Baenas, D.A. Moreno, C. García-Viguera, Selecting sprouts of Brassicaceae for optimum phytochemical composition, J. Agric. Food Chem. 60 (2012) 11409–11420, https://doi.org/10.1021/jf302863c.
[13] M. Francisco, D.A. Moreno, M.E. Cartea, F. Ferreres, C. García-Viguera, P. Velasco, Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable Brassica rapa, J. Chromatogr. A 1216 (2009) 6611–6619, https://doi.org/10.1016/j.chroma.2009.07.055.
[14] R. Dominguez-Perles, S. Medina, D.Á. Moreno, C. García-Viguera, F. Ferreres, Á. Gil-Izquierdo, A new ultra-rapid UHPLC/MS/MS method for assessing glucoraphanin and sulforaphane bioavailability in human urine, Food Chem. 143 (2014) 132–138, https://doi.org/10.1016/J.FOODCHEM.2013.07.116.
[15] J. Cramer, E. Jeffery, Sulforaphane absorption and excretion following ingestion of a semi-purified broccoli powder rich in glucoraphanin and broccoli sprouts in healthy men, Nutr. Cancer 63 (2011) 196–201, https://doi.org/10.1080/01635581.2011.523495.
[16] G. Paxinos, C. Watson, S. Diego, L. Boston, N. York, The Rat Brainin Stereotaxic, Coordinates Academic Press,, 1982.
[17] N. Baenas, I. Gómez-Jodar, D.A. Moreno, C. García-Viguera, P.M. Periago, Broccoli and radish sprouts are safe and rich in bioactive phytochemicals, Postharvest Biol. Technol. 127 (2017) 60–67, https://doi.org/10.1016/j.postharvbio.2017.01.010.
[18] J.W. Fahey, Y. Zhang, P. Talalay, Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens, Proc. Natl. Acad. Sci. 94 (1997) 10367–10372, https://doi.org/10.1073/pnas.94.19.10367.
[19] Z.-X. Gu, Q.-H. Guo, Y.-J. Gu, Factors influencing glucoraphanin and sulforaphane formation in brassica plants: a review, J. Integr. Agric. 11 (2012) 1804–1816, https://doi.org/10.1016/S2095-3119(12)60185-3.
[20] Q.T. Ostrom, M.A. Fahmideh, D.J. Cote, I.S. Muskens, J.M. Schraw, M.E. Scheurer, M.L. Bondy, Risk factors for childhood and adult primary brain tumors, Neuro. Oncol. 21 (2019) 1357–1375, https://doi.org/10.1093/NEUONC/NOZ123.
[21] A. Lombard, M. Digregorio, C. Delcamp, B. Rogister, C. Piette, N. Coppieters, The Subventricular zone, a hideout for adult and pediatric high-grade glioma stem cells, Front. Oncol. 10 (2021), https://doi.org/10.3389/FONC.2020.614930.
[22] L. Shu, D. Yu, F. Jin, Healthy dietary patterns, foods, and risk of glioma: a systematic review and meta-analysis of observational studies, Front. Nutr. 9 (2023) 1077452, https://doi.org/10.3389/FNUT.2022.1077452/BIBTEX.
[23] R.N. Auer, R.F.D. Maestro, R. Anderson, A simple and reproducible experimental in vivo glioma model, Can. J. Neurol. Sci. 8 (1981) 325–331, https://doi.org/10.1017/S0317167100043468.
[24] O. Warburg, The metabolism of carcinoma cells, J. Cancer Res. 9 (1925) 148–163, https://doi.org/10.1158/JCR.1925.148.
[25] M.G.V. Heiden, L.C. Cantley, C.B. Thompson, Understanding the warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029, https://doi.org/10.1126/SCIENCE.1160809.
[26] L.F. Barros, I. Ruminot, A. San Martín, R. Lerchundi, I. Fernández-Moncada, F. Baeza-Lehnert, Aerobic glycolysis in the brain: warburg and crabtree contra

pasteur, Neurochem. Res. 46 (2021) 15–22, https://doi.org/10.1007/s11064-020-02964-w.
[27] Y.Q. Tan, X. Zhang, S. Zhang, T. Zhu, M. Garg, P.E. Lobie and V. Pandey, Mitochondria: The metabolic switch of cellular oncogenic transformation, *Biochim. Biophys. acta. Rev. cancer*, DOI:10.1016/J.BBCAN.2021.188534.
[28] S. Liu, L. Dong, W. Shi, Z. Zheng, Z. Liu, L. Meng, Y. Xin, X. Jiang, Potential targets and treatments affect oxidative stress in gliomas: an overview of molecular mechanisms, Front. Pharmacol. 13 (2022), https://doi.org/10.3389/fphar.2022.921070.
[29] L.S. T. J. O'hare, L.E. Wong, Force, D.E. Irving, Glucosinolate composition and anti-cancer potential of seed-sprouts from horticultural members of the brassicaceae, Acta Hortic. 744 (2007) 181–188, https://doi.org/10.17660/ACTAHORTIC.2007.744.18.
[30] J. López-Cervantes, L.G. Tirado-Noriega, D.I. Sánchez-Machado, O.N. Campas-Baypoli, E.U. Cantú-Soto, J.A. Núñez-Gastélum, Biochemical composition of broccoli seeds and sprouts at different stages of seedling development, Int. J. Food Sci. Technol. 48 (2013) 2267–2275, https://doi.org/10.1111/ijfs.12213.
[31] C.A. Houghton, R.G. Fassett, J.S. Coombes, Sulforaphane: translational research from laboratory bench to clinic, Nutr. Rev. 71 (2013) 709–726, https://doi.org/10.1111/NURE.12060.
[32] A.E. Wagner, A.M. Terschluesen, G. Rimbach, Health promoting effects of brassica-derived phytochemicals: from chemopreventive and anti-inflammatory activities to epigenetic regulation, Oxid. Med. Cell. Longev. 2013 (2013) 1–12, https://doi.org/10.1155/2013/964539.
[33] M. La Marca, P. Beffy, C. Della Croce, P.G. Gervasi, R. Iori, E. Puccinelli, V. Longo, Structural influence of isothiocyanates on expression of cytochrome P450, phase II enzymes, and activation of Nrf2 in primary rat hepatocytes, Food Chem. Toxicol. 50 (2012) 2822–2830, https://doi.org/10.1016/j.fct.2012.05.044.
[34] T. Greco, G. Fiskum, Brain mitochondria from rats treated with sulforaphane are resistant to redox-regulated permeability transition, J. Bioenerg. Biomembr. 42 (2010) 491–497, https://doi.org/10.1007/S10863-010-9312-9.
[35] A. Tavakkoli, M. Iranshahi, S.H. Hasheminezhad, A.W. Hayes, G. Karimi, The neuroprotective activities of natural products through the Nrf2 upregulation, Phytother. Res. 33 (2019) 2256–2273, https://doi.org/10.1002/PTR.6427.
[36] D. James, S. Devaraj, P. Bellur, S. Lakkanna, J. Vicini, S. Boddupalli, Novel concepts of broccoli sulforaphanes and disease: induction of phase II antioxidant and detoxification enzymes by enhanced-glucoraphanin broccoli, Nutr. Rev. 70 (2012) 654–665, https://doi.org/10.1111/J.1753-4887.2012.00532.X.
[37] P.K. Dash, J. Zhao, S.A. Orsi, M. Zhang, A.N. Moore, Sulforaphane improves cognitive function administered following traumatic brain injury, Neurosci. Lett. 460 (2009) 103–107, https://doi.org/10.1016/J.NEULET.2009.04.028.
[38] L. Subedi, J.H. Lee, S. Yumnam, E. Ji, S.Y. Kim, Anti-inflammatory effect of sulforaphane on LPS-activated microglia potentially through JNK/AP-1/NF-κB Inhibition and Nrf2/HO-1 activation, Cells 8 (2019) 194, https://doi.org/10.3390/CELLS8020194.
[39] L. Guo, R. Yang, Z. Wang, Q. Guo, Z. Gu, Glucoraphanin, sulforaphane and myrosinase activity in germinating broccoli sprouts as affected by growth temperature and plant organs, J. Funct. Foods 9 (2014) 70–77, https://doi.org/10.1016/j.jff.2014.04.015.
[40] Y. Zhang, E.C. Callaway, High cellular accumulation of sulphoraphane a dietary anticarcinogen is followed by rapid transporter-mediated export as a glutathione conjugate, Biochem. J. 364 (2002) 301, https://doi.org/10.1042/BJ3640301.
[41] O.A. Elkashty, S.D. Tran, Sulforaphane as a promising natural molecule for cancer prevention and treatment, Curr. Med. Sci. 41 (2021) 250–269, https://doi.org/10.1007/s11596-021-2341-2.
[42] D. Gomez-Zepeda, M. Taghi, J.M. Scherrmann, X. Decleves, M.C. Menet, ABC transporters at the blood–brain interfaces, their study models, and drug delivery implications in gliomas, Pharmaceutics 12 (2020) 20, https://doi.org/10.3390/PHARMACEUTICS12010020.
[43] J.W. Fahey, K.L. Wade, K.K. Stephenson, A.A. Panjwani, H. Liu, G. Cornblatt, B. S. Cornblatt, S.L. Ownby, E. Fuchs, W.D. Holtzclaw, L.J. Cheskin, Bioavailability of sulforaphane following ingestion of glucoraphanin-rich broccoli sprout and seed extracts with active myrosinase: a pilot study of the effects of proton pump inhibitor administration, Nutrients 11 (2019) 1489, https://doi.org/10.3390/nu11071489.
[44] J.M.P. Ferreira De Oliveira, M. Costa, T. Pedrosa, P. Pinto, C. Remedios, H. Oliveira, F. Pimentel, L. Almeida, C. Santos, Sulforaphane induces oxidative stress and death by p53-independent mechanism: implication of impaired glutathione recycling, PLoS One 9 (2014) 92980, https://doi.org/10.1371/journal.pone.0092980.