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REVIEW

Chasing Graphene-Based Anticancer Drugs: Where are We Now on the Biomedical Graphene Roadmap?

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Abstract: Graphene and graphene-based materials have attracted growing interest for potential applications in medicine because of their good biocompatibility, cargo capability and possible surface functionalizations. In parallel, prototypic graphene-based devices have been developed to diagnose, imaging and track tumor growth in cancer patients. There is a growing number of reports on the use of graphene and its functionalized derivatives in the design of innovative drugs delivery systems, photothermal and photodynamic cancer therapy, and as a platform to combine multiple therapies. The aim of this review is to introduce the latest scientific achievements in the field of innovative composite graphene materials as potentially applied in cancer therapy. The "Technology and Innovation Roadmap" published in the Graphene Flagship indicates, that the first anti-cancer drugs using graphene and graphene-derived materials will have appeared on the market by 2030. However, it is necessary to broaden understanding of graphene-based material interactions with cellular metabolism and signaling at the functional level, as well as toxicity. The main aspects of further research should elucidate how treatment methods (e.g., photothermal therapy, photodynamic therapy, combination therapy) and the physicochemical properties of graphene materials influence their ability to modulate autophagy and kill cancer cells. Interestingly, recent scientific reports also prove that graphene nanocomposites modulate cancer cell death by inducing precise autophagy dysfunctions caused by lysosome damage. It turns out as well that developing photothermal oncological treatments, it should be taken into account that near-infrared-II radiation (1000–1500 nm) is a better option than NIR-I (750–1000 nm) because it can penetrate deeper into tissues due to less scattering at longer wavelengths radiation.

Keywords: graphene-based materials, oncological therapies, cancer treatment, biomedical innovations, drugs delivery systems

Introduction

Chemotherapy is currently one of the main methods of cancer treatment, but its effectiveness is limited due to systemic toxicity, suboptimal effectiveness and chemoresistance of cancer cells. For this reason, more selective therapeutic methods development is needed. These modern methods among others are based mainly on the use of materials that can act as carriers of anti-cancer drugs with unique biophysicochemical properties, especially in the area of binding xenobiotics and physiological metabolites, including absorption and penetration of biological membranes, volume of distribution, and therefore bioavailability and potential selectivity of the chemotherapeutic agent.[1–4] One of these types of materials that has recently been widely researched for use in oncological therapies is graphene. This material was invented in 2004, but the real race of scientists to use it commercially in various fields of science began a few years later, when it began to be produced in large quantities.

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Graphene (GN) is an allotropic form of carbon and, as a single graphite layer, it has recently been one of the most intensely investigated two-dimensional (2DM) materials. Graphene structure is based on a densely packed hexagonal crystal lattice one-atom thick. Carbon atoms are all sp^2 hybridized forming six-membered rings arranged in a kind of honeycomb lattice. The honeycomb structure of graphene having a thickness of one atom consists of soft equivalent subnets connected by σ bonds, each carbon atom having a relatively labile pair of π electrons contributing to a delocalized electron lattice. The labile π electrons are responsible for relatively higher electron density right below and above the 2D plane of the graphene monolayer. These electrons, by means of easy interaction with the boundary molecular orbitals of different organic compounds, to a large extent facilitate electrophilic substitution reactions as opposed to nucleophilic substitution ones. The planar structure of graphene also enables participation in various reactions, such as cycloaddition, click responses and carbene insertion reactions. Graphene has a hydrophobic character with the water contact angle in the range of 95–100°.[3–8] It is weakly dispersible in water and requires the use of surfactants or other stabilizing agents to form a suspension. In addition, it is distinguished by remarkable chemical stability, characterized by pronounced passivity in many chemical processes. Intensive research on the use of graphene and its derivatives in biomedicine pertains to their many unique physicochemical properties. The first one on the list of advantages is the high mechanical strength, which is two hundred times greater than that of steel of the same thickness (Young's modulus ~ 1000 GPa). It is a very hard material and surprisingly flexible at the same time. The C–C bond length in graphene is 1.42 Å and the crystal lattice constant is 2046 Å. Strong bonds between the C–C carbon atoms and the specific electronic structure of graphene ensure excellent electrical and thermal conductivity with a low coefficient of thermal expansion. Thus, graphene is characterized by high thermal (~5000 W/mK) and electrical conductivity, high mobility of charge carriers (200,000 cm^2/Vs at 300 K) and a specific capacity of 100 F/g. Moreover, it is characterized by high fracture toughness, which is 130 GPa. It was also shown that single-layer graphene transmitted 97.7% of total incident light over a wide range of wavelengths. Optical image contrast and light absorption increase with an increasing number of graphene layers. High light transmission, charge mobility and photoluminescence make graphene an important material for magnetic resonance imaging (MRI) and biomedical imaging applications. Another advantage of graphene is its large specific surface area (2630 m^2 / g) a. The list of advantages of this material also includes cheap and scalable production. Graphene is one of the thinnest and strongest materials in the universe. In terms of physical and chemical parameters, it is considered the most optimal nanomaterial.[6–10]

The graphene surface can be covalently and noncovalently modified by attaching different functional groups. The purpose of these modifications is to improve the dispersibility of graphene in water in order to facilitate optimal targeting of cancer cells as in oncology therapies. One of them is the reaction of graphene with oxygen. Graphene in the oxidized form (GO) is much easier to mix with water than regular graphene, which provides higher biocompatibility and bioavailability in the human body. The -COOH and -OH groups are both present in the oxidized form of graphene on the planar and edged structural motifs. All in all, hydrophilic GO groups include hydroxyl, carbonyl, carboxyl, and carboxylate functional groups which allow for enhanced interaction with proteins (eg, extracellular matrix proteins, growth factors, receptors) through electrostatic, covalent, and hydrogen bonds. It is known that such enhanced interactions between GOs and proteins significantly improve cell adhesion to the GO surface. Moreover, GO is highly adherent to the phospholipid bilayer of the cell membrane, inducing more efficient cell adhesion.[4,11–13] Thus, the phosphate and polar head groups that occur in the outermost layer of the cell membrane show a high affinity for the hydrophilic GO functional groups due to electrostatic interactions and hydrophilic properties. In addition, the oxidation process significantly reduces the electrical and thermal conductivity of graphene oxide (~2000 W/mK) compared to pure graphene (~5000 W/mK), which is a desired effect from the point of view of biomedical applications. However, in order to partially reproduce the original electrical properties of graphene, it is possible to perform a chemical reduction, ie removal of oxygen from graphene oxide or derivative materials produced from it. As a result, reduced graphene oxide (rGO) is obtained, being a material with a moderate degree of oxygen enrichment, which is an intermediate form between graphene oxide and unoxidized graphene. Thus, graphene, graphene oxide and reduced graphene oxide constitute three separate types of chemical materials of different physicochemical properties, which should not be treated as substitutes for one another. The mechanical resistance of GO is significantly lower (Young's modulus ~ 220 GPa) than that of pure

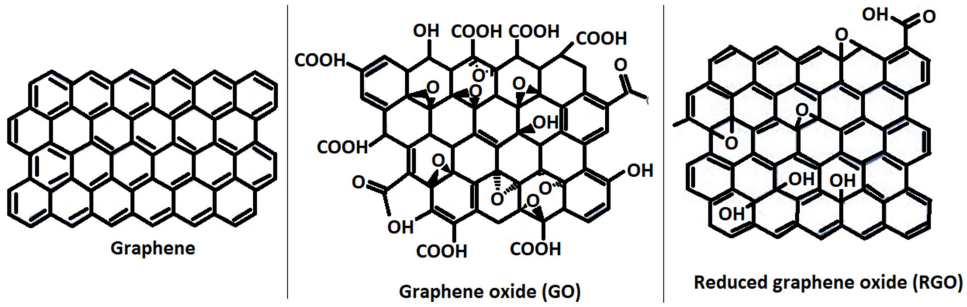

Figure 1 Structure of graphene, graphene oxide and reduced graphene oxide.

graphene. For example, nano-onions (GNOs) exhibit a modulus of elasticity of 32 GPa and a fracture strength of 120 MPa.[6–8,10–13]

Figure 1 shows the structure of graphene, graphene oxide and reduced graphene oxide. This article reviews the latest and most interesting scientific advances in the use of graphene specifically focusing on drug delivery technology and cancer therapies. In addition to that, this work also refers to the available literature on the effects of graphene preparations on various types of cancer cells (in vitro studies) as well as animal models of cancer (in vivo studies).

Methods

The selection of publications for this review manuscript takes into account the importance of scientific research on a given issue as a priority criterion. The most frequently searched keywords were: anticancer graphene therapy, graphene-based materials in oncology, graphene in oncology. Another key to the selection of cited publications was the use of the latest important scientific research that indicates areas for further exploration.

Graphene and Graphene Derivatives in Oncology

Graphene crosses physiological barriers and cellular structures by various routes of exposure or administration, entering the organism and individual cells, and ultimately causes different biological effects as observed both in vivo and in vitro.[14–16] Graphene is absorbed into cells depending on its physicochemical properties. Parameters such as shape, size, coating, charge, isoelectric point, hydrodynamic diameter, and pH gradient are important for allowing GO to pass through the cell membrane. Graphene nanoparticles with diameters <100 nm can relatively easily enter the cell, and those with diameters <40 nm can penetrate into different organelles including the nucleus. GO sheets can insert into the lipid bilayer, adhere to and wrap around the cell membrane, or become internalized into the cell.[11,17] On the other hand, larger protein-coated graphene oxide (PCGO) nanoparticles (~1 μm) enter cells mainly via phagocytosis, and smaller PCGO nanoparticles (~500 nm) enter cells mainly by endocytosis by means of clathrin coated vesicles.[13] It was shown that PEGylated reduced graphene oxide (PrGO) and rGO strongly adhere to the lipid bilayer of the cell membrane due to the interaction of hydrophobic, unmodified graphite domains with the lipid component of the cell membrane.[18] Therefore, it is concluded that prolonged exposure or too high concentration of graphene may cause biological or physical damage to the cell membrane, along with destabilization of actin filaments and the cytoskeleton. Current data show that GO sheets interact with the plasma membrane and are phagocytized by macrophages. Three major macrophage receptors are involved in phagocytosis of GNS: the mannose receptor (MR), the complement receptor (CR), and the Fcg receptor (FcgR). In addition, the FcgR is a needle receptor in the mediated phagocytic pathway.[19] In turn, the GO protein crown promotes the recognition of macrophages by receptors, especially IgG, which is located in the protein corona. Yue et al (2012) observed that macrophages undergo significant morphological changes after contact with GO.[20] Sydlik et al (2015) report that after internalization, graphene accumulated in the cell nucleus, cytoplasm and perinuclear space induced cytotoxicity in mouse macrophages by increasing intracellular generation of ROS. This was due to the reduction of inner mitochondrial membrane potential and also by triggering apoptosis via activation of the mitochondrial apoptotic pathway.[21] Moreover, it was proved by Nasiłowska et al (2020) that cancer cells exposed to graphene oxide on their own

may show viability reduction by up to 52.7%. These studies were performed on cancer cell lines MDA-MB-231 (neuroblastoma) and SW-954 (Vulvar Cancer). These tests were performed in two variants. In the first variant, graphene oxide was sprayed on a Petri dish, and then both cancer cell lines were cultured. The second variant of the study consisted of covering both types of cancer cells with an aerosol containing GO. In both study scenarios, tumor cell lines were incubated and then tested after 24, 48 and 72 h. Cell viability and surface morphology were measured in each of this three stages of the study. Well, the evaluation after 72 h showed that application of GO aerosol resulted in a significant decrease in cancer cell viability by 52.7% (MDA-MB-231) and 26.4% (SW-954), respectively, as compared to placebo.[22]

Current treatments of cancer include chemotherapy, surgery, and radiation therapy. The side effects of these treatments and their effects on organs or tissues are very well described and documented in the literature.[10,11,23–25] For this reason, new therapeutic agents are sought, which would show lower cytotoxicity, nephrotoxicity and higher targeted bioavailability than the currently used chemotherapeutic agents.[16,23,26,27] A relatively new area of research with great scientific potential is based on the use of graphene in anticancer therapies. Studies on new graphene oncological therapies, ie chemotherapy, gene therapy, photothermal and photodynamic therapy, are already available in the literature.[14,28–31] It should also be noted that the vast majority of research on the use of graphene in drug delivery technology concerns cancer chemotherapy. The high graphene-to-mass ratio and the hydrophobic π bond network enable covalent or non-covalent functionalization of graphene in order to increase the load of hydrophobic or aromatic compounds with anticancer activity.[7,8,32,33] A breakthrough in this regard was the report on the use of graphene oxide (GO) as an efficient nanocarrier for drug delivery, written by Dai et al in 2008. After this publication, there was a lot of interesting work done investigating the possibility of using graphene in drug delivery systems.[18] Graphene-based materials usually aggregate in an aqueous medium containing proteins, salts or other compounds, provided that they are chemically modified or in other words, functionalized. Functionalization of graphene influences the hydrophobicity and the surface charge by changing the degree of polarization and/or ionization which consequently changes the observed biological effects. For example, GO rich in carboxyl groups (-COOH), present with negatively charged surfaces at physiological pH. However, graphene derivatives may be modified by coupling reactions with amino groups (eg NH2-PEG-NH2), which give the surface a positive charge at physiological pH of 7.4. It is commonly believed that anionic particles are less toxic than cationic particles, while neutral particles are more biocompatible, mainly due to different affinities for negatively charged proteins and phospholipids.[11,28,34,35]

GO is more widely used than graphene for biomedical applications due to a wide range of reactions and functionalization opportunities. Therefore, for most biomedical applications, graphene and GO are functionalized covalently by hydroxyl, carboxyl or epoxy groups or noncovalently by interactions of the π surface of electrons, electrostatic interactions or hydrophobic interactions. GO has been functionalized with many biocompatible and water-soluble polymers such as polyethylene glycol (PEG), polyethylenimine (PEI), polyvinyl alcohol (PVA), chitosan, amino groups, amphiphilic copolymers, and sulfonyl groups. Interestingly, these functionalized GO conjugates proved to be effective adsorbents for hydrophobic drug molecules as well. This is due to their amphiphilic nature and the presence of oxygenated, unsubstituted graphene domains.[11,34–36] Chitosan-functionalized graphene oxide (ChrGO) nano sheets were produced by microwave assisted reduction using covalent GO functionalization. Such a solution was used in the nano-delivery of intracellular anti-cancer drug into the breast cancer cells.[37] Demirel et al (2020) reported that PEGylation of GO using PEG copolymer conjugated to multiple pyrenes, reduce GO to rGO and simultaneously increase the percentage of PEG on GO surface. In vitro tests showed that graphene with a PEG content greater than 75% and longer PEG chains exhibited higher biocompatibility and solubility than their counterparts with shorter PEG chains.[33]

Functionalizing GO with hyaluronic acid (HA) is also used to more effectively target GO into cancer cells. HA has high biocompatibility, biodegradability, and also shows strong binding affinity and selective targeting for tumor markers, especially cluster determinant 44 (CD44) and hyaluronan receptor (HARE). GO-HA has been coloaded with drugs such as doxorubicin DOX and paclitaxel (Ptx) and the obtained drug delivery system (GO-HA-DOX/Ptx) has been used for the efficient incapacitation of cancer cells.[38] Another dopamine (DA) functionalized drug delivery system was designed based on the nGO nanocarrier (DA-nGO). DA significantly improves the delivery of methotrexate (MTX) as an anticancer cargo into target cells.[39] Dendrimers and hyperbranched polymers, especially polyglycerol, are able to effectively improve the biofunctionality and biological properties of graphene sheets.[11,40]

The combination of functionalization possibilities with the use of multifunctionality of graphene and its derivatives enables the development of innovative drug delivery systems with controlled biological properties. Moreover, high-capacity drug loading graphene-polymer platforms have already paved the way for the development of new drug delivery systems for various biomedical applications. Therefore, it seems justified to design innovative drug transport systems where conventional graphene or common polymer would not be able to provide such high drug release efficiency.[29,30,41,42]

Zhou et al (2022) in their study applied smart and multifunctional MnO2-doped GO nanosystem for delivery of photosensitizer (Ce6) and cisplatin. As a result, the decomposition of hydrogen peroxide into oxygen was catalyzed to ease the hypoxia of the tumor, whereas the level of glutathione was reduced in targeted tumor cells. Then the Mn^{2+} cation was continuously generated for the progressive Fenton-like reaction, thus enhancing the antitumor effect. Additionally, hyaluronic acid was used as a surface modifier of the prepared nanosystem to improve its targeting properties. The ultimate result is increased toxicity against cancer cells and consequently inhibition of tumor growth.[43]

Molecular Mechanisms of Graphene's Interaction with Cancer Cells

The mechanism of the reduction of cancer cell viability mediated by graphene and graphene-based materials is, among others, attributed to an increase in the production of reactive oxygen species (ROS). ROS can cause nuclear and mitochondrial DNA damage leading to mitochondrial dysfunction, thereby reducing the viability of cancer cells. Thus, GO nanomaterials, by increasing the production of ROS and inducing DNA damage, can sensitize cancer cells to apoptosis. Reactive oxygen species are by-products of cellular metabolism, which are physiologically mainly formed in the mitochondrial respiratory chain. ROS is the collective term used to denote highly reactive oxygen derivatives, some of which are free radicals, including the hydroxyl radical ($^{\bullet}OH$), superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), the alkoxy radical ($RO^{\bullet}$),the peroxyl radical ($ROO^{\bullet}$) and singlet oxygen ($^1O_2$) and triplet oxygen ($^3O_2$). Under normal homeostatic conditions, ROS are produced in amounts that do not interfere with human body functioning. Quite the contrary, their role is often beneficial and helps regulate different metabolic processes. However, exposure to pro-oxidative factors causes increased production of ROS in cells, leading to oxidative stress. The result of this phenomenon is damage to DNA, proteins and lipids in normal cells of tissues and organs, as well as to cancer cells also those becoming resistant to drugs. The negative effects of ROS on the human body are controlled by antioxidant systems, exogenous antioxidants and endogenous antioxidants, which, working together with natural enzymatic oxygen defense mechanisms, protect cells against damage by free radicals. However, when the concentration of ROS is too high, the protective system cannot keep up with ROS removal. The body then fails to repair the structures that have been destroyed. The result of these processes is the formation of pathological changes, inflammation or the appearance of mutations in the genetic material of the cells. The consequence of this are functional and structural changes, which are the basis for the induction of tumor. Interestingly, according to the latest scientific reports, reactive oxygen species can lead cancer cells beyond the critical point, which results in the activation of cell death pathways and the reduction of tumor progression. Recent discoveries show that ROS under certain conditions can induce apoptosis and autophagy of cancer cells. Elevated levels of ROS in cancer cells can be used as a tool for selective therapeutic targeting on the cellular level. For this reason, ROS may be important for medical design of novel oncological therapies.[44–46]

It has been reported that GO induces cell cytotoxicity by inducing oxidative stress (ROS) and thus damaging cellular structures by attacking various biomolecules. These are mostly proteins, nucleic acids, unsaturated fatty acids, and carbohydrates in both eukaryotic and prokaryotic cells. Oxidative stress results from an imbalance between oxidants and antioxidants, resulting in disruptions of redox signaling and control. The presence of excessive oxidants causes damage to biomolecules.[47,48] For this reason, the effects of GO on oxidative stress in SKOV3 (*Ovarian serous cystadenocarcinoma)* cells were evaluated by (Gurunathan et al, 2022). In order to determine the influence of GO on oxidative stress in SKOV3 cells, the cells were treated with GO (25 µg/mL), C6-ceramide (15 µg/mL), cisplatin (6 µg/mL), and GW4869 (25 µM) and then incubated for 24 h. Various markers of oxidative stress were then measured, including ROS production, lipid hydroperoxide, isoprostanes, nitric oxide, malondialdehyde and carbonyl content in protein. After the incubation, the level of ROS in the treated cells was measured. Treatment of SKOV3 with GO, CIS (cisplatin) and (C6-ceramide) C6-Cer cells increased ROS production. SKOV3 cells treated with GO, CIS, and C6-Cer produced 25, 30, and 25 µmol

of ROS, respectively, while untreated cells produced 10 µmol ROS. It was also observed that GO (10–50 µg/mL), CIS (2–10 µg/mL) and C6-Cer (5–25 µM) inhibited tumor cell viability, proliferation and cytotoxicity depending on the concentration. Moreover, GO, CIS and C6-Cer were noted to stimulate the activity of neutral sphingomyelinase, acetylcholine esterase, total exosome protein concentration and exosome number, which is associated with increased levels of apoptosis, oxidative stress and endoplasmic reticulum stress. Human ovarian cancer cells secreted exosomes with typical cup-shaped morphology and surface protein biomarkers. Further, chemokine and cytokine levels were significantly higher in exosomes isolated from GO-treated SKOV3 cells than in those isolated from control cells. Furthermore, the expression levels of CD9, TSG101, CD81 and CD63 were significantly higher in GO-treated cells rather than in control cells. This study, therefore, further identifies GO as a potential tool for targeting the exosome pathway and stimulating exosome biogenesis and release.[49–51]

Furthermore, GO nanostructures can inhibit cancer cell metastasis and migration, and inhibit prostaglandin-mediated inflammatory responses. It is also assumed (Tian et al, 2017), that graphene oxide nanostructures can inhibit the invasion of cancer cells by disrupting the actin cytoskeleton. The A549 cell line (lung cancer) was used in the study. The results show that GO nanoparticles delay cancer cell migration by disrupting intracellular actin filaments. Consequently, A549 cells show slower migration and the structure of intracellular actin filament changes drastically. During the experiment, it was observed that GO nanosheets can absorb large amounts of actin and change the secondary structures of actin monomers.[52]

Depending on the conditions, GO nanoparticles can induce or inhibit autophagy in cancer cells. Molecular pathways, such as MAPK, ATG, JNK, and Akt, can be regulated by GO nanomaterials, leading to apoptosis and autophagy. By stimulating autophagy, GO nanocarriers can increase the sensitivity of cancer cells to chemotherapy. On the other hand, GO nanoparticles can enhance inflammation and reduce cell survival by impairing autophagy flux. Shen J. et al proved (2022) both in the in vitro and in vivo research protocols that GO significantly inhibited growth of colon cancer. It was possible because GO could relatively easily penetrate inside the HCT116 cells through endocytosis. During the study, tumor cells were treated with the medium containing 0.10 and 50 µg/mL GO for 24, 48 and 72 hours. GO treatment resulted in the production of reactive oxygen species (ROS), apoptosis, autophagy, cytotoxicity and activation of the AMPK/mTOR/ULK1 signaling pathway. Thus, graphene oxide has obvious anti-cancer effects, which seems to be mostly achieved by induction of autophagic death of HCT116 cells and activation of the ROS-dependent AMPK/mTOR/ULK1 pathway.[51,53]

Recently, Krętowski R. et al (2022) have proved that reduced graphene oxide (rGO) can stimulate autophagy and apoptosis in breast tumor cells, thus reducing tumor progression. In that study, there were two cell lines used: MDA-MB -231 (Breast adenocarcinoma) and ZR-75-1 (Invasive breast carcinoma of no special type). In order to analyze the molecular and cellular effects of rGO on breast cancer, MTT viability test, flow cytometry and Western blotting were used. Incubation of tumor cells with rGO produced a number of effects, including cell cycle arrest, stimulation of autophagy, and finally apoptotic cell death. It was also noted that apoptosis of cancer cells was accompanied by activation of caspase 9 and caspase 3, decreased mitochondrial membrane potential and dysregulated expression of mitochondrial proteins. These phenomena all prove that rGOinduced apoptosis via the intrinsic pathway. The antiproliferative effect of rGO was due to a decrease in MMP (matrix metalloproteinases), cell cycle arrest which was accompanied by deregulated p21, and increased autophagy in breast cancer cells. At the same time, it was observed that rGO had only minimal effect on normal human fibroblasts. In addition to that, it was observed that N-Acetylcysteine - an antioxidant (NAC) being an ROS scavenger, reduced the cytotoxic effect of rGO on the tested cancer cells. Moreover, the coexistence of autophagy and apoptosis was noted in MDA-MB-231 and ZR-75-1 cells exposed to rGO, which proves the multidirectional cytostatic and cytotoxic effects of rGO-derived material.[54]

Mbeh et al (2014) described the cytotoxicity of albumin functionalized GONRs (Graphene Oxide Nanoribons) against A549 cells. This allowed for the determination of the dose-dependency and related cytotoxicity of the compound. Albumin functionalized GONRs at a dose <50 µg/mL did not exhibit significant cytotoxicity, however incubation of A549 cells with higher dosage (100 µg/mL) resulted in the loss of cell proliferation and induction of apoptosis.[36] More recently, Chng et al reported a comparative study on the cytotoxicity of GONRs and GONPs (Graphene Oxide Nanoplatelets). In vitro cytotoxicity study focusing on a human lung cancer model (A549 cells) demonstrated that

GONRs induced a significantly higher cytotoxic response than GONPs at all of the dosage used (3–400 µg/mL). The increased cytotoxicity of GONRs is explained by the high GONRs shape factor and the presence of more carbonyl groups (28.22% for GONRs vs 11.06% for GONPs).[4]

The anticancer properties of graphene-based materials to some extent result from the induction of autophagy and/or the suppression of autophagic flux. The consequence of this fact is the accumulation of autophagic mediators (as LC3, and p62) that are involved in the apoptotic, necroptotic, and necrotic cell death of cancer cells, as well as the modulation of the anti-tumor immune response (Figure 2). During research, graphene-based materials have been observed to activate autophagy through oxidative stress/ER and MAPK or TLR or JNK or AMPK/mTOR/ULK1, ATG, and Akt signaling, but large graphene nanoparticles may also cause lysosomal dysfunction by blocking autophagic flux. At the same time, it is known that the autophagic mechanism occurs through the integration of various stress signals (eg oxidative stress, metabolic stress) by transcription factors and various protein kinases, including AMP-activated protein kinase (AMPK), protein kinase B/AKT, mitogen-activated kinase (MAPK), rapamycin mechanistic target (mTOR), nuclear factor and erythroid factor. Among many transcription factors and signaling molecules involved in the regulation of autophagy, these are of greatest importance for the modulation of autophagy by graphene-based materials. Autophagy initiation is started by transcription of autophagy-associated (ATG) genes, followed by highly orchestrated sequential activation of post-translationally modified ATG proteins organized into functional complexes. This leads to autophagosome nucleation and elongation, closure and finally fusion with the lysosome. In turn, autophagosome biogenesis occurs by recruiting the complex of kinase (ULK1, mammalian homolog of ATG1), ATG13, and a 200 kD family of adhesive kinase interacting protein to the phagophore assembly site. This ultimately leads to the generation and localization of the LC3-II autophagosome, which promotes the expansion of the autophagosomal membrane and its further closure and fusion with the lysosome. As a result, ubiquitinated cytoplasmic material bound to autophagic cargo receptors such as sequestosome 1 (p62) is ultimately degraded.[55–58]

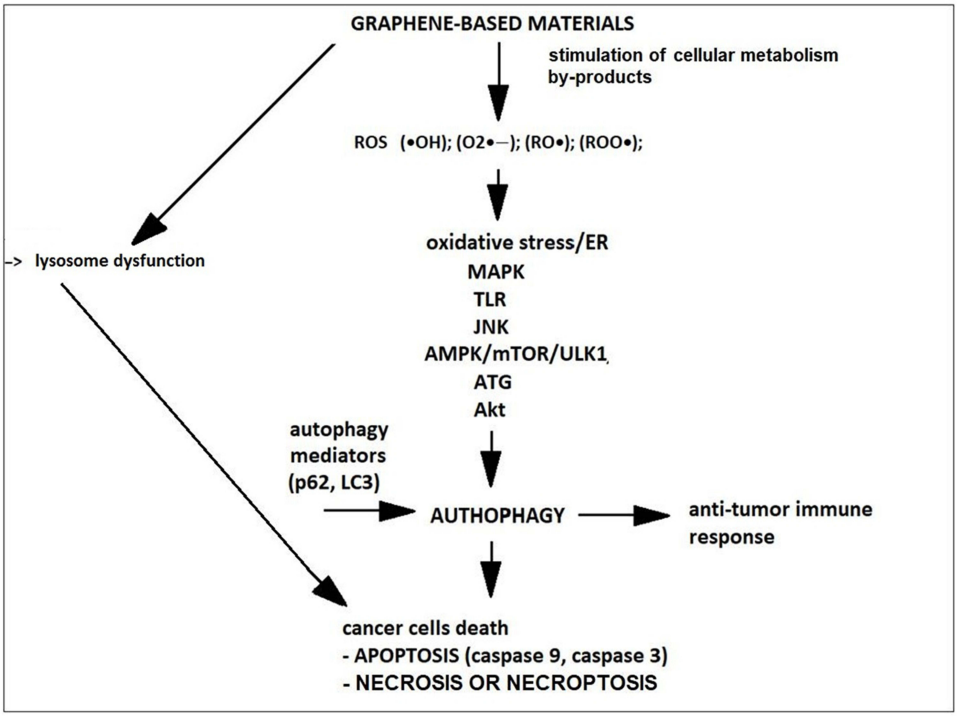

Figure 2 Molecular mechanisms of graphene's interaction with cancer cells. Graphene-based materials induce autophagy through oxidative/ER stress and MAPK, TLR, JNK, AMPK/mTOR/ ULK1, ATG, or Akt signaling, but can simultaneously block autophagic flux, causing lysosomal dysfunction. This leads to the accumulation of autophagic mediators such as LC3, p62, which are involved in the death of cancer cells.

Abbreviations: ROS, reactive oxygen species; ER, endoplasmic reticulum; MAPK, mitogen-activated protein kinases; TLRs, Toll-like receptors; JNK, c-Jun NH2-terminal kinase; mTOR, mechanistic target of rapamycin complex 1; ATG, autophagy-related; Akt, alpha serine/threonine-protein kinase; LC3, microtubule-associated light chain 3.

A deeper understanding of graphene-based materials – interestingly, interactions at a functional level – is necessary before these findings can be used to enhance the effectiveness of graphene materials as new anticancer drugs and drug delivery systems. The main aspects of further research should clarify how the treatment method (eg photothermal therapy, photodynamic therapy, combined therapy) and the physicochemical properties of graphene materials affect their ability to modulate autophagy and kill cancer cells, as well it should be validated in the in vivo conditions.

Autophagy is indispensable in normal cellular processes, yet detrimental to cancer treatment because it severely lowers therapeutic effectiveness. One of the keys to solve this issue may be the use of nanometric graphene structures affecting lysosomes. Liang et al (2023) developed a versatile nanoplatform based on manganese-doped graphene quantum dots (Mn-FGQDs) for precise and efficient photodynamic attenuation of lysosomes. Mn-FGQDs retain their high photodynamic efficiency in the lysosomal environment and are structurally robust. The incorporation of Mn facilitates their accumulation in lysosomes and strengthens the generation capability of reactive oxygen species (ROS). Light-triggered ROS production would primarily affect lysosomal function, leading to lysosomal impairment. The fact is that a continuous increase in the level of oxidative stress in lysosomes leads to the abnormal growth of autophagosomes and autolysosomes and, consequently, to serious autophagy dysfunctions. As a result, this leads to the death of cancer cells through precise autophagy dysfunction caused by lysosome impairment.[59]

The Use of Graphene Derivatives in the Controlled Release of Anticancer Drugs

Graphene oxide produced by the Hummers' method is an ideal nanocarrier for the efficient transport of various drugs. Mostly, a specific type of GO is used for this purpose, which usually consists of 1–3 layers (1–2 nm thick) and sizes ranging from a few to several hundred nanometers.

For the first time it was described in the publication by Liu et al (2008) referring to covalently functionalized polyethylene glycol (PEG) graphene as a delivery system for anticancer drugs. Modification of graphene with polyethylene glycol increased its solubility in water. The compound was additionally loaded with the camptothecin derivative SN-38 (a hydrophobic anticancer drug). Studies have shown an increase in the chemotherapeutic effectiveness by 2–3 orders of magnitude against HCT-116 colorectal cancer cells as compared to irinotecan (a water-soluble prodrug of SN-38). The shortcoming of this study was the fact that the capacity of the SN-38 drug on modified graphene in a limiting solvent (dimethyl sulfoxide) was only 0.1 g drug/g GO-PEG. This work showed that the release of the drug from the GO was pH dependent, suggesting the possibility of a controlled release of the drug depending on the environmental acidity.[18]

It has also been reported that fluorinated graphene oxide (FGO – fluorinated GO) modified with folic acid is an effective drug carrier. Functionalization of folic acid on GO is one of the more common methods used for targeted cancer therapy. Many types of cancer cells overexpress folic acid-binding proteins on the cell surface, including uterine, bone, colon, meningeal, ovary, and lymphocytic cancers. This material shows off the following properties: high loading capacity, water solubility, switchable fluorescence, controllable and pH-dependent drug release, as well as targeted drug delivery. By means of application of the designed oxygen introduction technique, the modified nano-sheets, ie FGO were used as targeting agent. Additionally, adapting the fluorinated graphene to the nano-scale structure based on sp^2 hybridized carbon atom lattice improved the observed photothermal performance. Doxorubicin (DOX) was also packed onto FGO-FA (FA – folic acid). The FA present in the material guarantees the targeted delivery of the nanoparticle to cancer cells presenting with the FA receptor, and then releases DOX in an acidic intracellular environment.[9] Other studies report the use of graphene as a specific hybrid to attach graphene compounds in order to form composite nanoparticles with increased drug-binding capacity, greater water solubility and the ability to control drug release as a result of physiological changes (ionic strength).[54,60,61] Lin et al (2013) proved that DOX, loaded on GO with functionalized folic acid, increases the cytotoxicity against OCM-1 human choroidal melanoma cells (viability <20%) whereas the DOX-GO hybrid induced significantly lower cytotoxicity (~40% viability) relative to control human ARPE-19 retinal pigment epithelial cells (after 24 hours of incubation). In addition, it has been shown to be able to load almost 100% DOX up to a concentration of 0.2 mg DOX/mg GO.[62]

In 2019, Zhang et al used histidine to mediate the hydrothermal expansion of coatings made of amorphous zinc oxide (a-ZnO) on gold nanoparticles (Au-His@a-ZnO). Au-His@a-ZnO nanoparticles were integrated by using a carbodiimide

cross-linker into the PEGylated graphene oxide (PEG-GO) plane. As a result, nanocomposites Apt@GO@Au-His @a-ZnO@DOX (Apt stands for aptamer) were obtained, which showed a high transport capacity of doxorubicin (DOX). The metal-drug complex dissociated in such a way as to release the anti-cancer Zn^{2+} ions in an environment with a relatively low pH (typical for cancer cells). Moreover, the study also demonstrated the high biostability of the obtained nanocomposites and their ability to attach to lung cancer cells by means of aptamers. Moreover, using A549 cells (human lungs adenoma cells), significant targeting of drug delivery and high in vivo efficacy of Apt@GO@Au-His @a-ZnO@DOX nanocomposites were demonstrated in a mouse model.[63]

It has also been reported that graphene oxide was loaded with doxorubicin and poly (allylamine) functionalized with citraconic anhydride. In this case, the pH-responsive polyelectrolyte released DOX at a lower endosomal pH. A 53% higher release efficiency was observed within 24 hours, when the developed compound penetrated into endosomes of neoplastic cells at a pH within the range of 5.0–6.5.[64] In turn, another researcher developed reduced graphene oxide functionalized by polyethyleneimine (PEI)-GO with covalently coupled β-cyclodextrin as a DOX carrier for pH modulated drug delivery. Cyclodextrins are rings of glucose molecules that have the ability to form stable inclusion complexes with a wide variety of compounds without the need for covalent bond formation. This is possible by the formation of a hydrophobic internal cavity by cyclodextrins, which allows for the encapsulation of other hydrophobic molecules and drugs such as DOX, thereby increasing their loading capacity.[65]

Itatahine et al (2018) described a camptothecin delivery system (CPT) that was developed on the basis of magnetic nanoparticles deposited by means of mesoporous silica on layered graphene oxide. Such carriers had a loading drug capacity of 20% and showed controlled drug release depending on the pH. It was found in the study that the designed structures were highly effective against HeLa cells (HeLa – cervical cancer epithelial cells). In addition, it was found that having appropriate magnetic properties and accompanying high adsorption capacity, the material was a versatile nanoplatform for cancer chemotherapy using magnetic targeting.[7]

Another article describes a synthesized nanocomposite of carboxymethylcellulose hydrogel (CMC) and graphene quantum dots (GQD) with improved in vitro swelling capacity. The material is pH sensitive and makes acidic conditions of the medium optimal for DOX release. This interesting property has been used in studies where the nanocomposite was used as the theranostic nano factor for K562 cancer cells (myelogenous leukemia cell line).[30] In a similar study, GQD cross-linked chitosan was loaded with a model drug (sodium salicylate) and encapsulated in CMC. In the gastrointestinal tract, this nanocomposite showed high stability and excellent controlled drug release.[37] Another elaboration, focusing on the fluorescent properties of GQD, describes DOX-GQD-RGD nanocomposite (RDG - a peptide sequence consisting of the amino acids arginine, glycine and aspartic acid). This graphene-derived material was developed for screening real-time cellular uptake along with observation of drug release as a function of time.[66]

In the work by Sumathra et al (2018) designed nanocomposites of graphene oxide (GO) with hydroxyapatite (HAP) and chitosan (CS), loaded with the drug cisplatin (CDDP) were described. These compounds were used in the treatment of bone cancer. They were aimed at inhibiting the development of osteosarcoma cells and increasing the growth of osteoblast. The conclusion from the research is that GO/HAP / CS-3/CDDP nanocomposites showed high cytotoxicity against cancer cells, while maintaining high viability of osteoblast-like cells.[16] Another publication describes the use of modified graphene oxide in the treatment of breast cancer. The modification is based on attaching camptothecin (CPT) and magnetic nanoparticles to the surface of reduced graphene oxide, which was cross-linked with a 4-hydroxycoumarin (4-HC) photosensitizer through an allylamine (AA) linker. It was observed that the obtained MrGO-AA-g-4-HC, which was loaded with CPT, showed higher toxicity toward breast cancer cells compared to healthy cell lines, thus selectively causing death of cancer cells.[63] Another study describes methyl acrylate-modified graphene (GO-g-MA) additionally conjugated with folic acid. The obtained GO-g-MA/FA nanomaterial was packed with an anticancer drug – paclitaxel (PTX) through hydrophobic interactions and π-π bonds. The GO-g-MA/FA-PTX carrier showed off cytotoxic effect in 39% (as a cell viability), which was effective in inhibiting the growth of breast cancer cells and reducing the tumor size. Moreover, this system was effective in alleviating mitochondrial dysfunction of breast cancer.[64] Another innovative research work was devoted to increasing the penetration of bioactive materials during chemotherapy. Protein-polymer carriers were designed specifically for this purpose. Graphene oxide was functionalized with egg white (OVA) and

polymethyl methacrylate and then loaded with doxorubicin. As a result, the designed OVA-PMMA-GO-DOX system showed higher effectiveness and better controlled drug release.[67]

Photothermal and Photodynamic Cancer Therapy

Graphene (due to electromagnetic absorption) is also used in photodynamic therapy as a carrier of the so-called photosensitizers, whose task is to transmit energy to pathological tumor mass – heat generation – PTT (Photothermal Therapy). GN has also found application in photodynamic therapy, which is based on the production of reactive oxygen species – PDT (Photodynamic Therapy). Photodynamic therapy (PDT) is a relatively new cancer treatment that combines the use of oxygen, light, and the photosensitizer (PS) to generate highly cytotoxic reactive oxygen species (ROS) that aim to cause cancer cell death. Photosensitizers absorb incident light and generate free radicals, which react with local tissue to further produce reactive oxygen species and consequently cause cancer cell death. In the PDT process there are two types of mechanisms involved (type I and type II). Type I process in most cases requires the transformation of triplet oxygen in the ground state ($^3O_2$) into highly reactive singlet oxygen ($^1O_2$). In contrast Type II mechanism is based on the abstraction of electrons or hydrogen atoms by excitation of photosensitizers from the substrate. The first type of PDT process mechanism, unlike type II, is limited by the concentration of O2.[26,28,47,68–71] Photosensitizers are covalently or non-covalently functionalized on graphene nanoparticles. Porphyrin-based macrocyclic photosensitizers, including hemachrome and protoporphyrin, have routinely used in PDT, and are now extended to chlorins, phthalocyanines and other macrocyclic porphyrin-like compounds. Graphene nanoparticles have been studied to improve the existing limitations of macrocyclic photosensitizers, including poor tissue specificity and low water solubility.[24,31,54,68–72] Zhou et al (2011) reported the effects of graphene oxide carrier loaded with hypocrellin-A, a hydrophobic non-porphyrin photosensitizer, by non-covalent interactions at concentrations as high as 1 mg/mL. HeLa cells treated with this complex showed little or no dark toxicity. After 1 min. of photo irradiation, an increase in cytotoxicity of HeLa cells by about 10% was observed at drug concentrations of 1.65 μg/mL as compared to control with hypocrellin-A at the same concentration inducing PDT in cancer cells.[13] Zinc oxides have been shown to induce cancer cell death through the formation of singlet oxygen as induced by ultraviolet radiation. However, ultraviolet radiation significantly reduces tissue penetration and nonspecifically induces DNA damage. Hu et al showed (2013) that folic acid-GO-zinc oxide conjugates reduce the viability of HeLa cells by 80% at concentrations of 75 μg/mL. This is mainly the result of the activation of apoptotic pathways under the influence of 15-minute exposure to visible light (48.6 J cm^{-2}).[61]

The limitation of photodynamic therapy (PDT) is the fact that most photosensitizers (PS) have low ROS generation efficiency, which affects the therapeutic effect of PDT. This raises some need for the development of new PSs for PDT to improve its clinical relevance. A new strategy is to synthesize PS by modifying graphene quantum dots (GQDs) on the surface of upconversion nanoparticles doped with rare earth elements (UCNPs). This is to produce UCNP@GQD with a core-shell structure, which was actually done by Li et al (2022). Complex UCNPs @ GQD were easily prepared and showed excellent biocompatibility. Cytotoxic ROS were successfully generated after irradiation with near-infrared (NIR) light. This method provides a new paradigm with highly integrated functions that shows excellent prospects for PDT development.[73]

Materials for Photothermal Therapy (PTT) are designed to absorb electromagnetic energy and facilitate local temperature rise (hyperthermia) in order to ablate the nearby cells. Graphene has the ability to absorb visible and near-infrared radiation, thus causing a hyperthermic effect on cells and tissues.[30,68–71] Marković et al (2011) compared the in vitro efficacy of carbon nanotubes and GOs in PTT for targeted cancer cell treatment. It has been noted that at different exposure times ranging from 0.5 to 5 minutes, the concentration of nanoparticles needed to obtain the IC50 value is an order of magnitude lower for GO (4.1 μM) as compared to carbon nanotubes (49.3 μM).[14] In turn, Abdelsayed et al (2010) reported that about 16% of the excitation energy delivered by the laser beam (532 nm wavelength) was converted by GO into hot deionized water. This effect is mainly attributed to the deoxidation of GO.[68]

The first in vivo study of PTT using graphene was conducted in 2010 by Yang et al.[71] In vivo fluorescence imaging of the intravenously administered PEG-graphene-Cy7 molecular fluorophore showed high tumor uptake due to the EPR effect. Low retention of this material was noted in the reticuloendothelial systems of mice with tumors injected intravenously with a dose of 20 mg / kg body weight. In turn, Salaheldin et al (2019) showed that the G / Fe_3O_4

nanocomposite was highly effective in converting light into heat and is a novel, promising candidate for cancer therapy. HepG2 (liver cancer cell line) was treated with 400 μg/mL G / Fe_3O_4 for 24 hours. After the experiment, changes in cell morphology were noted, which revealed the toxic effect of the composite on the cellular DNA. A photothermal effect was observed for G / Fe_3O_4 after irradiation of HepG2 cells. In contrast, there was no expression of caspase-3 mRNA after 24 hours of cell exposure, suggesting involvement of the internal caspase-independent apoptotic pathway. Tumor cell viability decreased significantly after treatment with 10 and 50 μg/mL G / Fe_3O_4 from 40% to 5% after 48 hours of treatment.[70]

High photothermal conversion efficiency was achieved using a bovine serum albumin-modified rGO-based system used as a carrier. This system constituted a nanoplatform for zeolitic imidazolate framework-8 (BSArGO@ZIF-8) for PTT and combinatorial ion interference (IIT). It is an efficient source of Zn2+ that can disrupt intracellular homeostasis, leading to an increase in the level of ROS, mitochondrial damage and cells apoptosis. In this study, the viability of Cal27 (Tongue Squamous Cancer) cells after BSArGO@ZIF-8 NS and NIR (near-infrared) irradiation was approximately ~20%. A similar effect was achieved with analogous irradiation of SCC25 cells, where cell viability decreased to ~15%.[74]

Interestingly, most research in the field of photothermal therapy focuses on the use of laser light from the NIR-I window (usually 808 nm) to induce hyperthermia. However, it is worth emphasizing that biological systems lack chromophores that absorb radiation in this range, therefore in the treatment of deep or large cancerous tumors, the depth of its penetration into body tissues is limited. For this reason, the NIR-II window is a better option because it can penetrate deeper into tissues due to less scattering at longer radiation wavelengths. Additionally, longer wavelengths have less energy per photon. Therefore, when designing new methods for photothermal cancer treatment using graphene-based materials, researchers should consider the NIR-II region for irradiation (1000–1500 nm).[75–79]

Interestingly, PTT and PDT can be combined to achieve even more effective anticancer therapy. The photothermal effect (PTT), in addition to generating heat and destroying cancer cells, can be enhanced by the action of reactive oxygen species as induced by PTD.[30,31,68,70] Sherlock et al (2011) proved that low-energy photothermal heating up to about 43°C, showed a significant improvement in clathrin-dependent endocytosis in carbon nanotubes and graphite structures.[27] Sahu et al (2013) used hydrophilic methylene blue (US FDA approved) as a photosensitizing agent with GO. The study demonstrated complete ablation of HeLa tumor xenografts by combining the effects of PDT and PTT with no cancer remission within 15 days (in athymic mice). During the same period, mice treated with PTT or PDT alone, showed significant tumor regrowth, up to 30% of the relative volume (higher as compared to the group treated with PDT + PTT).[69]

Similarly, scientists have designed a complex of hybrid nanoparticle graphene oxide and iron oxide (FVIOs-GO) in the domain of a ferrimagnetic vortex as an efficient MTD agent. The FVIOs-GO nanoplatform has been proven to exhibit high thermal conversion efficiency and has been shown to generate significantly higher ROS levels in an alternating magnetic field (AMF). Both in vivo and in vitro tests revealed that amplified ROS generation was the dominant factor in provoking a strong immune response at a physiologically tolerable temperature below 40°C in the hypoxic tumor microenvironment. Due to the dual action of the ROS-related immunological effect and the magnetothermic effect, an impressive systemic therapeutic efficacy as observed in vivo, was achieved at a relatively low dose of 3 mg Fe/kg with two AMF treatment sessions, as compared to MTT (high dose 6–18 mg/kg with four to eight treatment cycles). Thus, the immune effect mediated by ROS makes it possible to further design new cancer magnetotherapies with significantly improved antitumor capabilities than those having been used today.[80]

Summary and Future Perspective

Graphene-based materials exhibit exceptional mechanical properties, high chemical and thermal stability, and radiation resistance, which make them a promising material in biomedical applications.[81–83] Moreover, these compounds show clinical potential as multifunctional platforms for new, effective and selectively targeted anticancer treatment. The results of in vitro and in vivo studies prove that formulations based on graphene can be widely used in medicine as multi-functional delivery agents. However, in order to use the potential of graphene in medical therapies, a solution must be found to several challenges that arise after this material enters the body's circulatory system. It has been proven that these materials accumulate in the cells and tissues of the body, which can cause toxic reactions leading to cell apoptosis and body dysfunction when the accumulated amount exceeds a certain limit.[16,83,84] Studies in mice have shown that

intravenous administration of GO leads to pulmonary edema, while intraperitoneal injection of GO causes its massive accumulation in the liver and spleen. Nevertheless, GO show good biocompatibility with red blood cells, resulting in a significantly longer circulation time in the body compared to other nanomaterials.[84–86] The toxicity of graphene-based materials may be influenced by various factors, including their physical state, chemical composition, size and surface charge.[87] Although GO and rGO have shown high biocompatibility in various studies, it should be taken into account that their toxicity may increase with subsequent functionalization. Therefore, the best solution is to functionalize graphene materials using biomimetic molecules, especially those that have received FDA approval.[75,88]

Recently, scientific reports have appeared that the biocompatibility and cytotoxicity of graphene-based materials largely depend on the interaction between the material and the body's immune system. It has been proven that the morphology of graphene and types of functional groups increasing its dispersibility in water, may be the key aspects in the development of optimal drug transportation systems for effective oncological therapies. Yang et al showed that the accumulation of nanographene sheets in the liver can be reduced by functionalizing them with PEG and that the material can be gradually removed from the body through metabolism without causing significant harm to the health of mice. Overall, functionalized nanosystems based on graphene materials show insignificant adverse effects on healthy cells, high selectivity, higher local therapeutic uptake and better drug biodistribution.[86–95] A promising concept would be to integrate graphene anticancer therapies with graphene imaging capabilities. For example, Raman imaging could facilitate tracking and monitoring the biodistribution and removal of the therapeutic load. Kim et al (2020) reported the possibility of regulating the cellular microenvironment, using graphene derivatives or graphene hybrid materials to effectively control cell differentiation and function, and consequently biological responsiveness of cancer cells.[61] Interestingly, the effect of graphene particle size on cell viability have not been much examined by researchers. Another important issue is the diffusion of particles through biological membranes, which can act as barriers and limit the penetration of graphene structures. An important step toward improving the stability and bioactivity of graphene-based drugs was the use of natural polyphenols in combination with graphene-based nanocomposites. Sathishkumar et al (2020) developed silver and gold nanoparticles hybridized with reduced GO nanocomposites and the anticancer flavone chrysin with improved bioactivity, biocompatibility and stability. These nanohybrids enhance cytotoxicity against breast cancer cell lines with low toxicity toward normal cells.[96]

In the context of designing cancer therapies using the photodynamic effect, recent scientific reports also prove that graphene nanocomposites modulate cancer cell death by inducing precise autophagy dysfunctions caused by lysosome damage. When developing photothermal cancer treatment methods, it should also be taken into account that near-infrared II radiation (1000–1500 nm) is a better option than NIR-I (750–1000 nm) because it can penetrate deeper into tissues due to lower scattering in the case of radiation with a longer wavelength.[75,97]

Table 1 Various Recent Reports and Research Results on the Use of Graphene-Based Materials (GBM) in Anticancer Therapies

GBM	Cancer Type/ Cell Line	Conclusions from the Experiment	Reference
GO/CisPt/Ce6@MH	MDA-MB-231 and RLE-6TN	Enhanced chemo-photodynamic therapy with tumor-targeted drug delivery. The key element of the nanosystem was the MnO2 surface doping, which was systematically characterized by TEM, AFM, XPS and elemental mapping.	[42]
GO	SKOV3	GO-stimulated exosome secretion in SKOV3 cells is associated with loss of cell viability and proliferation, oxidative and ER stress, increased cytotoxic, immunomodulatory effects, and a decrease ATP levels and the mitochondrial membrane potential. The expression levels of CD9, TSG101, CD63, and CD81 significantly increased in GO-treated cells. These findings suggested that GO has significant effects on exosome composition and biogenesis.	[48]
GO	HCT116	GO exerts anticancer effects against Colorectal cancer via ROS-dependent AMPK/mTOR/ULK-1 pathway-related autophagy and apoptosis.	[51]

(*Continued*)

Table 1 (Continued).

GBM	Cancer Type/ Cell Line	Conclusions from the Experiment	Reference
rGO	MDA-MB-231, ZR-75-1 and fibroblasts cells	RGO can induce cytotoxicity in MDA-MB-231 and ZR-75-1 cell lines but not in human skin fibroblasts. The antiproliferative effect of rGO is associated with a decrease in MMP, increased autophagy and cell cycle arrest in breast cancer cells.	[53]
Mn-FGQDs	-	Mn strengthens the generation capability of reactive oxygen species (ROS) and also facilitates its accumulation in lysosomes. Moreover, Mn-FGQDs are structurally robust and retain their high photodynamic efficiency in the lysosomal environment.	[59]
CPT-MrGO-AA -g-4-HC	MCF-7 and WS-1	The cytotoxic effects of CPT and 4-HC loaded carriers show higher toxicity effect against the human breast cancer cell line (MCF-7) compared with the normal fibroblast cell line (WS-1).	[98]
UCNP@GQD	HeLa	This new type photodynamic therapy (PDT) produced ROS efficiently under near-infrared light excitation. UCNPs@GQDs exhibited high biocompatibility and obvious concentration-dependent PDT efficiency, shedding light on nanomaterials-based PDT development.	[73]
BSArGO@ZIF-8 NSs	HeLa and SCC25 c	BSArGO@ZIF-8 NSs can promote cell apoptosis by initiating bim (a pro-apoptotic protein)-mediated mitochondrial apoptotic events, up-regulating PUMA/NOXA expression, and down-regulating the level of Bid/p53AIP1. Zn2+ excess triggers cellular dysfunction and mitochondria damage by activating the autophagy signaling pathways and disturbing the intracellular environmental homeostasis.	[74]
FVIOs-GO	4T1	Such a FVIOs-GO nanoplatform was shown to have high thermal conversion efficiency, and it was further proved to generate a significantly amplified ROS level under an alternating magnetic field (AMF). As a result of the dual action of magnetothermal effect and ROS-related immunologic effect, impressive in vivo systemic therapeutic efficacy was attained at a low dosage of 3 mg Fe/kg with two AMF treatments, as compared to that of MTT (high dosage of 6–18 mg/kg under four to eight AMF treatments)	[80]
GOQD, C18-GVFHQTVS, C18P	Choroidal Neovascularization	MMP9-responsive GOQD-based minocycline-loaded nano-in-micro drug delivery system (C18PGM) is developed by chemically bonding GOQDs to an octadecyl-modified peptide sequence (C18-GVFHQTVS, C18P) that can be specifically cleaved by MMP9. Using a laser-induced CNV mouse model, the prepared C18PGM shows significant MMP9 inhibitory activity and anti-inflammatory action followed by antiangiogenic effects.	[82]
ChR@Ag-rGONCs and ChR@Au-rGONCs	MDA-MD-468 and MDA-MD-231	The presence of noble metal NPs on the rGO surface improved its thermal stability and performances in diverse physiological conditions. Moreover, the surface passivation of the fabricated NCs with natural anticancer flavone ChR improved its biocompatibility. Due to the synergistic impact of the plasmonic metal–rGO hybrids, the fabricated NCs exhibited an increased cytotoxic effect over that of free-ChR against two different breast carcinoma (MDA-MD-468 and MDA-MD-231) cell lines	[96]

A summary of the latest research results on the use of graphene-based materials (GBM) in anticancer therapies, which provide important information in the design of commercial therapeutic methods, is presented in Table 1.

The Graphene Flagship Internet portal reports that in the field of imaging and diagnostics, ultrafast graphene-based laser devices have been developed to diagnose and track tumor growth in cancer patients. The use of graphene may also

help to enhance the bioavailability of highly effective cancer drugs. All these new concepts for the support and improvement of cancer therapy, naturally require lots of research and clinical trials before they reach practical applicability in clinical practice. A detailed understanding of the molecular mechanisms of graphene's interaction with cancer cells may be crucial in the development of an effective graphene-based anti-cancer drug.[99–101]

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Disclosure

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