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Nanomedicine: pharmacological perspectives

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Abstract

Rapid developments in the field of nanotechnology are generating enormous interest in prospecting the potential of multifunctional therapeutic nanovectors in the field of personalized medicine. Although the nanomaterials are of same dimensions to many cellular machineries, their interaction with cells, tissues and organs of the body are not well understood. This in turn forms the rationale for a comprehensive study of these nanoplatforms in various disease models in terms of their toxicity, pharmacokinetics, pharmacodynamics, and pharmacogenetics. Such a study will significantly aid in our quest in translating its application from bench to bedside.

Keywords: nanomedicine; pharmacodynamics; pharmacogenetics; pharmacokinetics; toxicity.

1. Introduction

Nanotechnology pertains to the understanding and control of matter generally in the 1–100 nm dimension range. A basic and conventional definition of nanotechnology would be engineering of functional systems at a molecular scale encompassing the concepts and advances made in this field. The widespread application of nanotechnology to medicine, known as nanomedicine, is based on a bottom-up concept, involving the use of precisely engineered materials of this dimension to develop novel therapeutic and diagnostic modalities [1, 2]. It has an enormous potential to improve healthcare, particularly in cancer management [3].

The ability of nanomaterials to immobilize specific ligands on the surface makes them ideal candidates for molecularly sensitive detection, efficient contrast agents for molecular imaging, carriers for targeted drug as well as gene delivery, and therapeutic reagents for targeted photothermal therapy. To date, a wide number of nanoparticle-based therapeutic and diagnostic agents have been developed for the treatment of a variety of highly morbid and mortality causing diseases such as cancer, cardiovascular diseases, diabetes, and other immunomodulatory disorders including rheumatoid arthritis, asthma, and different allergic conditions [4, 5]. In the past two decades, computer-based algorithms have extensively aided our search for novel tumor-specific molecular targets [6], and simultaneously innovative drug delivery systems [7, 8] with due emphasis on site specific targeting aimed at improving the efficacy of the drugs on a spatial and temporal scale. One of the caveats associated with untimely diagnosis and inefficient delivery of effective therapeutic regimens for solid tumors such as breast, prostate, lung, and gastrointestinal cancers has been due to predominant use of poorly predictive preclinical models [9]. The use of nanoparticle-based drug delivery systems for developing site specific tumor targeting therapies represent the emergent strategies to combat the menace of these highly morbid and mortality causing diseases in the past decade [10].

Cells, the building block of any living organisms, have dimensions in the order of 10 μm. However, the constituent components of the cell including subcellular organelles as well as DNA, RNA, and proteins are much smaller and are in the submicron size domain. Even some of the structural proteins have a dimension of just 5 nm, which is comparable to the dimensions of smallest manmade nanoparticles. This analogous size comparison has enabled us to use nanoparticles as molecular probes capable of inspecting the cellular machinery without introducing too much interference [11].

Nanotechnology represents a new facet of combinatorial science based upon the premise and concepts interfacing nanomaterials and biological systems. The “nano-bio” interface encompasses kinetics and thermodynamic exchanges, dynamic physicochemical interactions between nanomaterial surfaces along with their associated biological components. It includes phospholipids, membranes, proteins, endocytic vesicles, organelles, DNA, RNA, and biological fluids. Viruses exemplify the classic example of natural nanoparticles with a core-shell structure. The core encloses infectious agents that can control the transcription and translation machinery of the host cells. The shell is made of various proteins or proteins embedded in lipid membranes. These virus-based nanoparticles have been extensively used as gene delivery vehicles due to their high gene transfection efficiency [12, 13]. This technology has been developed with the hope of achieving site specific stable drug delivery systems with marginal side effects to combat the menace of highly morbid and mortality causing degenerative diseases such as cancer and diabetes. Another advantage considered by the proponents of this

technology refers to the development of cost-effective applications such as development of diagnostic kits or therapeutic drug regimens, which involve the concepts from different realms of sciences.

The basic unit of the nanoplatform-based therapeutic regimen is a nanoparticle. The unique ability of the nanoparticles to deliver cargos to the subcellular compartments makes it a wonderful carrier which is commonly referred to as nanovectors. In the present study, we have tried to summarize and characterize the abilities of different types of nanovectors having constituent lipid units such as liposomes to polymeric nanoparticles having divergent applications such as delivering cargos to subcellular organelles such as mitochondria towards the management of debilitating neurological mitochondriopathies such as Parkinson's on the one hand to the delivery of short interfering RNA (siRNA) on the other hand for modulating the keystone signaling pathways playing an integral role in the management of highly morbid and mortality causing diseases such as cancer. Micelles constitute another class of nanovectors which are capable of being functionalized to cross the blood-brain barrier (BBB), whereas dendrimers could absorb and carry any type of cargos in their cavities through hydrophobic interactions in the treatment of not only cancer but also autoimmune diseases. Apart from the above-mentioned types of nanovector inorganic nanoparticles such as metallic or ceramic, nanoparticles also have enormous potentials to be used as nanovectors in the management of a variety of debilitating diseases such as diabetes and cancer. Last but not the least, carbon nanotubes have emerged as a novel nanovector being extensively used in the formulation of novel diagnostic kits aiding in the detection of a variety of cancers at its nascent stages as well as in the development of novel cancer management strategies. One of the prerequisites for optimal utilization of these wide ranges of nanovectors mentioned above pertains to their pharmacokinetic and pharmacodynamic properties which essentially determine their stability and efficacy in biological systems. A combination of both attributes plays a key role in determining the pharmacogenetic potential of the drug delivery systems in the management of diseases such as cancer which have extremely divergent sources of origin and function. The following sections in the present review will aim to highlight and summarize the divergent attributes of the various classes of nanovectors with their prospective roles in disease management.

2. Nanovectors

Nanovectors, commonly named "nanopharmaceuticals or nanomedicines", have been designated by the European Science Foundation to be nanometer size scale complex systems, consisting of a core constituent material, a therapeutic and/or imaging agent, along with biological surface modifiers, which significantly enhance the biodistribution and dispersive patterns of nanoparticles upon site specific targeting of tumors [14]. Nanovectors are a type of nanoplatforms which are so especially chemically engineered that they can be loaded with drugs, targeting agents such as an antibody, imaging agent, and many more to facilitate their biomedical application. A major clinical advantage of nanovectors over simple immunotargeted drugs is the target specific delivery of therapeutic drugs on a spatial and temporal scale. Targeting methods that have been investigated range from covalently linked antibodies [15, 16] to other mechanisms based on the size and physical properties of the nanovectors [17]. Figure 1 displays the most common nanoconstituents used to build nanovectors. Nanovector formulations are considered to reduce the clearance time of small peptide drugs, offer fortification of active agents from enzymatic or environmental degradation, and avoid barriers to the targeting of the active moiety. Examples of such barriers include the protective exclusion by the BBB or the vascular endothelium; the amplified osmotic pressure states in cancer environments, resulting in outward flow of therapeutic moieties [18] and nanoparticle sequestration by the reticuloendothelial system (RES) [19, 20].

Many polymer-based nanovectors have been explored [15, 21, 22] and some seem to be promising candidates for clinical trials. Figure 1 shows the divergent classes of nanoparticles having unique properties to have potential in being prospected as putative nanovectors. The stability and efficacy of a nanovector in a biological system is the determinant factor for its successful implementation in formulation of novel therapeutic regimens for a variety of disease managements. These attributes solely depend upon their pharmacokinetic and pharmacodynamic properties. Together, all of these attributes contribute to the pharmacogenic potential of a drug delivery system, which could be appropriately modulated to cater to the needs of personalized medicine.

2.1. Liposomes

Liposomes are widely used nanovectors because of their easy preparation, acceptable toxicity, biocompatibility profiles, and commercial availability [23]. They are small vehicles with an aqueous inner core enclosed by unilamellar or multilamellar phospholipid bilayers [24, 25]. Liposomes have been widely used as pharmaceutical carriers in the past decade because of their unique abilities to: (i) encapsulate both hydrophilic and hydrophobic therapeutic agents with high efficiency; (ii) protect the encapsulated drugs from undesired effects of external conditions; (iii) be functionalized with specific ligands that can target specific cells, tissues, and organs of interest; (iv) be coated with inert and biocompatible polymers such as polyethylene glycol (PEG), in turn prolonging the liposome circulation half-life *in vivo*; and (v) form desired formulations with needed composition, size, surface charge, and other properties [26, 27]. Conventional liposomes have relatively modest transport capacity across the BBB [28, 29] and rapid RES clearance. These issues have been largely overcome by using liposomal surface coatings such as PEG and maintaining the particle diameter at <100 nm. These modifications reduce liposome aggregation and their recognition by the RES [30, 31]. PEGylated liposomes can also be manufactured to increase their access through the BBB by receptor or absorptive-mediated transcytosis. The latter can be achieved by coating the liposome surface with monoclonal

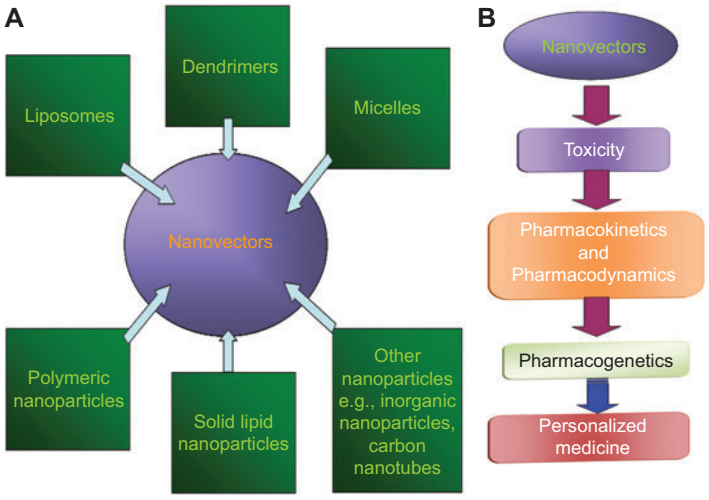

Figure 1 (A) Nanoparticles as nanovectors: nanovectors are the principal constituents of nanopharmaceuticals, which have immense applications in the field of personalized medicine. The figure essentially depicts the divergent classes of nanoparticles with unique photophysical attributes being prospected as nanovectors. (B) Nanovectors in personalized medicine: the failure of current therapeutic regimens in providing solutions to problems affecting the management of highly debilitating and mortality causing diseases such as cancer has lead to the establishment of a novel therapeutic regimen of personalized medicine. A key to prospecting the putative nanopharmaceuticals in personalized medicine is dependent upon its stability and efficacy in biological systems. The stability and efficacy of nanopharmaceuticals in biological systems are governed by their pharmacokinetic and pharmacodynamic properties, whereas toxicity patterns of the nanopharmaceuticals are dependent upon its size as well as its physicochemical properties. The toxicity patterns along with the pharmacodynamic and pharmacokinetic properties of nanopharmaceuticals determine its pharmacogenetic potential. All of the above-mentioned attributes need to be accounted before prospecting nanopharmaceuticals in personalized medicine.

antibodies to glial fibrillary acidic proteins (GFAPs), transferrin receptors or human insulin receptors [25, 32]. The ability of the liposomes to deliver anticancer agents to the brain might indeed be prospected for releasing neurotrophins and growth factors in the injured regions of brain to facilitate its repair and regeneration [33]. Liposomes have also been used experimentally to deliver genes to the brain. A plasmid expressing tyrosine hydroxylase, a key enzyme involved in the biosynthesis of dopamine, was delivered to the dopamine-depleted striatum of the rat using systemically administered PEG immunoliposome nanoparticles coated with transferrin receptors. The plasmids were expressed throughout the striatum and the nanoparticle delivered gene normalized tyrosine hydroxylase expression levels [32].

In addition to its use as a carrier for drugs and genes, liposomes have also been extensively exploited as nonviral siRNA delivery systems. There are various commercially available cationic lipid formulations that efficiently bind to negatively charged cell membranes and improve the transfection efficiency of siRNAs in cultured cells [34, 35]. Liposomes have also been exploited for *in vivo* delivery of siRNAs [36, 37]. Cationic liposomes containing siRNAs, which are often called solid nucleic acid lipid particles (SNALPs), have been mostly stabilized by PEGylation. The attachment of PEG has successfully improved the targeting efficiency and stability of siRNAs in the blood circulation, and due to this modification, a significant reduction of their rate of renal clearance has been achieved. SNALPs have productively inhibited apolipoprotein B gene expression in the livers of mice and nonhuman primates, which resulted in a reduction of serum cholesterol and low-density lipoprotein (LDL) levels [38]. Maximizing the amount of siRNA that could be encapsulated by a lipid complex enabled the total amount of vehicle to be reduced to approximately one-third, which could potentially reduce the toxic effects of this treatment on the liver [39]. It has also been demonstrated that liver-specific siRNAs can induce the reduction of proprotein convertase subtilisin/kexin type 9 (PCSK9) mRNA levels by 50–70% in mice and rats, and of human PCSK9 mRNA levels in transgenic mice by more than 70% [40], as a treatment for hypercholesterolemia. Vitamin A-coupled liposomes have been used to deliver anti-gp46 siRNAs to fibrogenic hepatic tissues in the treatment of liver cirrhosis [41]. SNALP formulations have also been used to facilitate the delivery of siRNAs as a part of curative strategies against Ebola virus [42].

Apart from the above-mentioned applications, liposome-based nanocarriers have also been extensively used to deliver cargos to subcellular organelles such as mitochondria. MITO-Porter is well known as a liposome-based carrier system and has been extensively used for delivery of macromolecules into mitochondria via membrane fusion [43]. The Harashima group from Hokkaido University, Japan, have reported that the MITO-Porter could deliver cargos into the mitochondrial matrix, which contains the mtDNA pool [44]. Very recently, a dual function MITO-Porter has been developed, which possesses mitochondria-fusogenic inner and endosome-fusogenic outer envelopes. It is capable of penetrating mitochondrial membranes as well as endosomal membrane via stepwise

membrane fusion [45] and can target the mitochondrial genome [46]. Thus, the dual function MITO-Porter presents a novel nano-based platform facilitating the delivery of cargos to subcellular fractions of both cytoplasm and mitochondria. It might be pertinent to mention here that development of such novel nanoplatforms might find applications in the treatment of neurodegenerative mitochondriopathies [47].

2.2. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) constitute another form of nanovectors that are used for preparing solid lipid matrices stabilized by surfactants. These nanoparticles have diameters between approximately 50 and 1000 nm. General constituents of SLN consist of solid lipid(s), emulsifier(s), and water. In this case, a wide variety of lipids have been explored including triglycerides (e.g., tristearin), partial glycerides (e.g., imwitor), fatty acids (e.g., stearic acid), steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate) [48]. The addition of emulsifiers provides stabilization to the lipid dispersion. These types of nanovectors are easy to prepare and possess the characteristic attributes of nanomaterials in terms of their low cytotoxicity and good physical stability and, therefore, have the inherent ability to protect labile drugs from degradation as well as provide sustained and controlled drug release [49, 50]. Brioschi et al. have conclusively demonstrated the efficacy of solid lipid nanoparticles for the delivery of several antineoplastic agents *in vivo* in rat models of glioma [51]. These nanovector systems are capable of enhancing brain uptake of a wide variety of compounds including HIV protease inhibitors such as atazanavir [52].

Cationic solid lipid nanoparticles (cSLN), reconstituted from natural components of protein-free LDL, seems to be a very promising nanovector to deliver siRNA [53]. For example, Tristearin solid lipid nanoparticles, loaded with siRNA, showed sustained release of siRNA over a period of 10–13 days when administered by intradermal injection in mouse footpads [54]. Recently, Yu et al. tested the efficacy of cSLNs for co-delivery of paclitaxel (PTX) and human MCL1-specific siRNA in xenograft tumor of human epithelial carcinoma in mice and concluded that this conjugation significantly reduced the growth of the xenograft tumors [55].

Glioblastomas constitute one of the most aggressive types of neoplasms with extremely poor patient outcomes. Recently, Jin et al. demonstrated that use of the PEGylated c-Met siRNA-SLN complex significantly inhibited c-Met expression to ensure effective suppression of tumor growth without showing any adverse side effects in the orthotopic model of the U-87MG xenograft tumor model [56].

2.3. Polymeric nanoparticles

Polymeric nanoparticles are nanoparticles which are composed of polymers. They can be subdivided as nanospheres and nanocapsules. They are solid carriers ranging from 10 nm to 1000 nm in diameter prepared from natural or artificial polymers, which are mostly biodegradable. In these nanovectors, therapeutic drugs can be adsorbed, dissolved, entrapped, encapsulated, or covalently linked [57]. The process of making the carmustine polymer essentially involves the conversion of a biodegradable polyanhydride polymer to small polymer discs containing the alkylating agent bis(2-chloroethyl) nitrosourea (BCNU) which, in turn, are administered into the brain following the surgical removal of the tumor. With time, they slowly degrade to deliver the drug locally on a sustained basis to prevent the recurrence of recalcitrant tumors. The synthetic materials used to prepare polymeric nanoparticles consist of poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly[lactide-co-glycolide] (PLGA), poly(alkylcyanoacrylate) (PACA) and polyanhydride poly[bis (p-carboxyphenoxy)] propane-sebacic acid (PCPP-SA). In addition to the above-mentioned list, natural polymers such as chitosan, alginate, and gelatin have also been tested [58, 59]. When systemically administered, these nanoparticles are generally more stable than liposomes but are edged out by liposomes due to their poor pharmacokinetic properties, uptake by RES and along with their inability to cross the BBB. They are also like liposomes, coated on the surface with various target molecules to not only increase their BBB permeability but also significantly improve their pharmacokinetic properties [60], to facilitate targeting for delivery and imaging purposes. PLGA nanoparticles [61, 62] have been successfully used to deliver drugs for the treatment of neurodegenerative disorders. Nkansah et al. reported that the use of a PLGA nanosphere of 315 nm dimension can increase the retention of ciliary neurotrophic factor, hence improving its effectiveness [62]. In another report, pilocarpine-loaded PLGA nanoparticles were found to be unique in terms of the therapeutic effect of ophthalmic drug delivery with enhanced bioavailability and pharmacological response [63].

The first practical use of polymer therapeutics as anticancer agents occurred in the 1990s through the use of poly (styrene-co-maleic acid/anhydride) neocarzinostatin (SMANCS) [64, 65] and PEGylated proteins. In contrast to SMANCS, which is administered locally, PEG-protein conjugates [66] are administered parentally and are therefore useful for treating a wide variety of diseases. PEGylation has since been applied to various proteins, including enzymes, cytokines, and monoclonal antibody fragments. Asparagine (Asp) is an amino acid that is necessary for tumor growth. It is known that L-asparaginase depletes Asp and is active against acute lymphoblastic leukemia (ALL) and lymphoma. The only adverse side effect associated with its administration is appearance of symptoms associated with anaphylactic shock and other hypersensitivity reactions. Host antibody synthesis can also lead to premature asparaginase clearance from circulation. PEG-L-asparaginase (Oncaspar®) was the first nanoparticle based drug to obtain US Food and Drug Administration (FDA) approval in 1994 to treat ALL [67, 68].

Polymer-based vehicles have become attractive candidates for both systemic and local delivery of siRNAs [69, 70]. The positive charge of cationic polymers allows them absorb sufficient amounts of protons into the endosomes, which are maintained at a pH of 5.5–6.0. This is known as the proton-sponge effect. The process ensures lysosomal swelling and disruption of the endosomal membrane, which in turn facilitates the

transport of siRNAs into the cytosol [69]. Polyethyleneimine has been extensively used as a carrier of siRNAs and other nucleic acids. Moreover, polyethyleneimine can have either a linear or branched form with molecular weights ranging from 1 kDa to >1000 kDa. Because of this, the polymer structure can be optimized for effective delivery of specific agents [71, 72].

Involvement of nanotechnology currently has not only significantly enhanced the efficacy of polyethyleneimine-based siRNA delivery system but also substantially reduced its toxicity. For example, amphiphilic polyethyleneimine-based core-shell nanoparticles of 120 nm size show considerable potential as carriers for gene delivery [73]. As a result, the cytotoxicity of polyethyleneimine was found to be much reduced. Immobilization of polyethyleneimine onto poly(methyl methacrylate) solid support exhibited cytotoxicity three times lower than native polyethyleneimine. To achieve this, Wu et al. synthesized the polyethylene glycol-polyethyleneimine-SiRNA nanocomplex, which is considered a promising nonviral carrier for altering gene expression in the treatment of gastric cancer. This composite not only provided relatively high gene transfection efficiency but also showed low cytotoxicity [74]. In another study, this group also reported the potency of the gelatin-polyethyleneimine core-shell nanogel to be very effective for the siRNA target to the human argininosuccinate synthetase 1 (*ASS1*) gene in HeLa cells [75]. Apart from the above-mentioned examples, PLGA-based nanoparticles have also been used as delivery vehicles for siDCAMKL-1 inducing a targeted inhibition of *DCAMKL-1*, resulting in induction of key tumor suppressor microRNAs (miRNAs) in colon cancer cells both under *in vitro* and *in vivo* conditions [76].

2.4. Micelles

Polymeric micelles have been documented as one of the most promising carrier systems for drug delivery [77, 78]. They are known to form spontaneously in aqueous solutions of amphiphilic block copolymers and have core-shell architectures. These systems are capable of holding large amounts of drugs in their core protecting them from degradation, and their chemical composition, size, and morphology can easily be modified. The surface of polymeric micelles can also be functionalized so that they can pass through the BBB. Perhaps most importantly, there are wide varieties of therapeutic molecules that can be used with these materials including drugs, proteins, oligonucleotides, and imaging agents.

Vehicles, which are based on block copolymers of two or more polymer chains with different hydrophobicity, are composed of two or more covalently linked polymers with various physicochemical properties. They spontaneously assemble into a core-shell micellar structure in an aqueous media with a diameter of 20–100 nm to minimize the free energy of the system. The design of the vehicle is optimized for each drug or nucleic acid to be carried by adjusting the molar ratio of the block copolymer mixtures.

Specifically, the hydrophobic blocks form the core to minimize their exposure to aqueous surroundings, whereas the hydrophilic blocks form the corona-like shell to stabilize the core through direct contact with water [79]. This micellar structure plays a pivotal role in making it an ideal drug delivery nanovector system. Its hydrophobic core is capable of carrying pharmaceuticals, especially poorly soluble drugs, with high loading capacity (5–25% weight). Polymeric micelles have hydrophilic outer shells composed of PEG, which enables their extended retention in circulation, excellent enzymatic tolerance, and minimal nonspecific interactions with plasma proteins and blood cells [78, 80]. These characteristics also minimize accumulation of the drug in the RES. Moreover, polymeric micelles can easily pervade tumors with leaky vasculature to reach the interior/core of the target tissue [65, 81, 82].

In addition to their larger carrying capacity, micelles can also deliver drugs in a more controlled manner. The encapsulated drugs attached to the hydrophobic core of the micelles are released through bulk erosion of the biodegradable polymers, diffusion of the drug through the polymer matrix, or polymer swelling followed by drug diffusion. Some external conditions such as changes of pH and temperature can also trigger drug discharge from polymeric micelles [1, 83]. The enhanced stability of polymeric micelles in blood due to its higher critical micelle concentration value in comparison to other liposomal carriers makes it a suitable carrier to facilitate the delivery of drugs with varying solubility gradients [84]. The above-mentioned aphorism is strengthened by several studies where attempts have been made to modify the surface of these micelles with ligands such as antibodies, peptides, nucleic acid aptamers, carbohydrates, and small molecules, resulting in differential delivery and uptake by a subset of targeted cells, which will not only increase their specificity and efficacy but also reduce their systemic toxicity [85, 86]. Poly(D,L-lactic acid), poly(D,L-glycolic acid), poly(e-caprolactone), and their copolymers are also widely used biodegradable polymers capable of forming micelles for drug delivery and controlled release [79, 86, 87].

Hydrophobic and electrostatic interactions between charged block copolymers and oppositely charged macromolecules, such as nucleotides, permit the formation of nanoparticles with a core and outer shell structure, termed polyion complex micelles [77–88]. These micelles have several advantages over the conventional carrier systems (e.g., liposomes) including simple preparation, efficient inclusion of the therapeutic molecules without the need for chemical modification, and controlled release of their contents [88, 89]. A new carrier system has been developed for siRNAs and peptides uses PEG and poly-L-lysine (PLL) block copolymers with cross-linking disulfide bonds [77, 90–93]. Furthermore, both PLL and PEG are substances that are generally recognized as safe by the FDA and, in fact, PEG is already being used to develop sustained-action drugs such as PEGylated interferons [91]. The safety of vehicles prepared using these agents may further facilitate their development for use in a variety of highly morbid and mortality causing diseases [92, 93].

Although more studies are needed, polymeric micelles made up of poloxamer block copolymers (Pluronics®) and those with TAT-poly(ethylene glycol)-block-cholesterol are

now opening new avenues for the delivery of drugs, including those that are aimed to promote repair and/or regeneration of brain tissues [94].

Paclitaxel (PTX), an antimicrotubule agent, has a wide spectrum of antitumor activity including ovarian, breast, stomach, lung, and head and neck cancers [95–97]. NK105 which is a PTX-incorporating "core-shell type" polymeric micellar nanoparticle formulation has gone through clinical trials [93]. Although cisplatin has enormous clinical usefulness, due to its nephrotoxicity and neurotoxicity, it is found to be difficult to continue its administration on a temporal scale. Introduction of polymeric micelles through the polymer-metal complex formation between polyethylene glycol poly(glutamic acid) block copolymers and cisplatin (NC-6004) significantly enable the nanopolymer-conjugated drug to overcome this challenge many fold [98].

2.5. Dendrimers

Dendrimers have materialized as another promising platform for drug delivery because of their well-defined structural design and novel characteristics [99, 100]. Dendrimers are synthetic polymers consisting of an initiator core and multiple layers with active terminal groups. They are found to be globular with extensive branching. These layers comprise repeating units and each layer is called a generation. The core of a dendrimer is referred to as generation zero. The multivalent surfaces of dendrimers enable them to carry various drugs through covalent conjugation or electrostatic adsorption. On the other hand, dendrimers can absorb drugs using the cavities in their cores by hydrophobic interaction, hydrogen bond, or chemical linkage. In a recent report, it has been shown that attaching of a folate group as the targeting molecule in therapeutic agents such as methotrexate made the polyamidoamine-based G5 dendrimer approximately 10 times more potent than methotrexate alone in inhibiting tumor growth. Moreover, the targeted, methotrexate-loaded dendrimer had reduced systemic toxicity than free methotrexate [99]. Polyamidoamine dendrimers have drawn significant attention as promising drug delivery systems and extensive studies are being undertaken to tune its structure as well as molecular weights to optimize accumulation in tumors and thereby significantly enhancing its therapeutic efficacy.

Several dendrimers have been used for drug delivery purposes [101, 102]. For example, Witvrouw et al. reported that sulfonated polyamidoamine (PAMAM) dendrimers inhibited HIV replication by binding to gp120 glycoprotein located at the HIV surface [103]. Recently, a novel nanovector synthesized from five acylated PAMAM dendrimer conjugated to glycidol and folic acid as targeting moiety with fluorescein isothiocyanate (FITC) as fluorescent probe as well as methotrexate (an antimetabolite and antifolate drug) has been used in treatment of cancer and autoimmune diseases [104–106]. This dendrimer-based platform was found to be tremendously versatile enabling modification of the parent drug by switching from methotrexate to paclitaxel [107]. Its targeting mechanism involving internalization and cytotoxic behavior patterns tested under *in vitro* conditions on KB cells (a human epidermal carcinoma) and were found to be promising at relatively low concentration (50 nM). Association of RGD ligands [108] which are tripeptides based on arginine-glycine-aspartate enable a flourishing anticancer therapeutic regimen called antiangiogenic therapy, based on the detection, targeting, and prevention of neovascularization [109].

Dendrimer-boron conjugates are promising agents for boron neutron capture therapy (BNCT) of cancer. There is a large body of evidence documenting the development of successful dendrimer-based BNCT agents which meets the required criteria of low toxicity and high uptake by tumor cells, a maximum BNCT concentration in tumor cells with a high tumor/blood partition ratio (44:1) and rapid clearance from blood and healthy tissues with persistence in tumor cells [110]. Although its potential has not been realized completely, its progress is still very promising. This is further affirmed by the observations published from the Morrder group, which has developed polylysine dendrons bearing a high boron payload (using eight dodecaborane groups), having a dansyl-based fluorescent probe to allow monitoring, a PEG tail to improve the solubility in water, and an antibody for targeting have proven to be good candidates for this purpose, due to optimal synergistic actions of all the components of the nanosystems [111]. The use of G2 and G4 PAMAM dendrimers as scaffolds [112] for the grafting of Na(CH3)3NB10H8NCO on their surface has also been described. Another approach consisting of the targeting of endothelial cells of the tumor vasculature rather than the tumor itself has also been reported by the Barth group [113].

2.6. Other nanovectors

Apart from the above-mentioned types of nanovectors, recent investigations have revealed the existence of another class of nanoparticle-based platforms comprising biopolymers and their self-assemblies including albumin, polysaccharide, and virus. This novel aspect of this nanoparticle-based platform has opened up a new therapeutic modality with multiple applications. This is affirmed by the observations in numerous studies where the stability, efficacy, and biodistribution of small molecule drugs was significantly improved upon conjugation with human serum albumin [114, 115] or a polysaccharide such as chitosan [116, 117].

Inorganic nanoparticles such as metallic nanoparticles and ceramic nanoparticles have also attracted some attention for prospective therapeutic potentials. Iron oxide [118, 119] is one very promising metallic nanoparticle, being explored as a passive or targeting agent after being coated with dextran, surfactants, phospholipids, or other compounds to improve their stability. In a recent study, aminosilane-coated iron oxide nanoparticles were exploited as part of thermotherapy to treat brain tumors. Using magnetic field-induced excitation of iron oxide superparamagnetic nanoparticles, thermotherapy was observed to prolong the survival time significantly (4.5-fold) over controls in a rat tumor model [118]. In the study, tumors were developed by implantation of RG-2 cells into the brains of 120 male Fisher rats. Gold nanoparticles represent another class of metallic nanoparticles, which have good physical

and chemical properties, and thus represent ideal candidates for high infrared phototherapy [120]. Ceramic nanoparticles such as silica, titanium, and alumina with bioinert properties porous structures [121, 122] have recently been proposed as putative drug delivery vehicles to deliver drugs as part of various cancer therapy regimens.

Carbon-based nanoparticles such as functionalized carbon nanotubes (CNTs) and modified C_{60} fullerenes have emerged as attractive targets for development of novel emergent therapeutic platforms due to their widespread use in electronics and, most recently, in biological systems [123, 124]. Ever since their discovery in 1991 by Iijima [125], CNTs have drawn enormous attention due to their unique physicochemical and pharmacokinetic properties. They are now being prospected as novel nanoplatforms for the development of the next generation of targeted drug delivery systems [126–128]. The fact that CNTs could be engineered for labeling and targeting molecules, nucleic acids, peptides, proteins or drugs, to transport various molecules to specific target cells [129, 130] strengthens its stature as a novel class of nanovectors. Most low-molecular weight platinum anticancer drugs have short blood circulation times. As a consequence, tumor uptake and intracellular DNA-binding efficiency of these drugs are found to be reduced. To meet this challenge, a platinum(IV) [Pt(IV)] drug containing a folate derivative (FA) at an axial position has been attached to an amine-functionalized single-walled CNT (SWCNT). This particular surface engineering facilitated improved release of cisplatin upon intracellular reduction of Pt(IV) to Pt(II) [129]. The fullerene family, and especially C_{60}, has also been explored extensively in the biophysical and biomedical domains. This delivery system has been conjugated with several molecules including anticancer drugs (e.g., Taxol®) [131]. It has been demonstrated that close to 40 fullerenes can be accommodated onto a single skin cancer antibody named ZME-108, which has been used to administer drugs directly into melanomas [131].

3. Toxicity

One of the most predominant criteria that needs to be addressed prior to the use of nanoparticle-based drugs for clinical trials is the toxicity profiles of the prodrug combination. An accepted fact in this paradigm pertains to the unpredictable nature of toxicity patterns associated nanomaterials-based drugs due to their multicomponent nature, novel structures, and polydispersity. Sometimes the individual constituent of these multicomponent drugs might significantly contribute to its toxic effects due to the pharmacokinetic properties of these materials. This may be a consequence of unexpected interactions with target and nontarget tissues as well as with the organs involved in clearance. As summarized by Lanone and Boczkowski, the main molecular mechanism of *in vivo* nanotoxicity is the induction of oxidative stress by free radical formation. In excess, free radicals would significantly enhance damage to biological components through oxidation of lipids, proteins, and DNA and thereby inducing a systemic, inflammatory response through the upregulation of redox sensitive transcription factors (e.g., NF-κB), activator protein-1, and kinases involved in inflammation [132].

Intracellularly, nanomaterials may interact with subcellular components to disrupt or significantly alter cell function, or generate reactive oxygen species (ROS). Interactions of nanomaterials with the mitochondria and cell nucleus are being considered as main sources of toxicity. Unfried et al. postulated that nanomaterials such as silver-coated gold nanoparticles, fullerenes, block copolymer micelles, and CNTs might localize to mitochondria to induce apoptosis and ROS formation. They also hypothesized that nanomaterial-induced nuclear DNA damage, cell-cycle arrest, mutagenesis, and apoptosis could also be a possible source of toxicity [133]. Although still under debate, nanomaterials have been putatively suggested to be involved in the upregulation of free radical generating oxidases such as NADPH oxidase and xanthine oxidase, which are potent sources of free radicals in macrophages and neutrophils [132]. Apart from this, toxicity from the nanomaterials could also be due to their interaction with the surrounding microenvironment. When introduced or absorbed into the systemic circulation, its interaction with blood components could potentially cause hemolysis and thrombosis. Additionally, nanomaterial interactions with the immune system have been known to increase immunotoxicity as reviewed by Dobrovolskaia and McNeil [134]. Recently Lanone and Boczkowski stated that in liver metabolic modification of nanomaterials by their interaction with cytochrome P450 might be the predominant reason for enhanced hepatotoxicity due to generation of ROS [132]. The occurrence of systemic inflammation in clearance organs such as liver and kidney due to formation of reactive intermediates together with lack of biodegradation of some components of nanoparticle-based drug systems is the main cause of concern to the general public regarding the possible environmental and ecological consequences of widespread nanomaterial uses [135–137]. Results from different studies have confirmed that the toxicity profiles of nanomaterial-based drugs are in the same range of their components (some of which are well-known cytotoxic agents). To achieve this, these systems, in many cases, are designed to convert the biomolecules such as cytotoxic agents to their less toxic states by altering their delivery and clearance. Therefore, it seems reasonable to set the threshold for tolerance and therapeutic index of the developing nanoconjugate drugs to the same levels being currently used for therapeutic regimens in vogue based on anticancer agents. One of the biggest edifying factors contributing to the acceptance of nanoconjugate-based therapeutic drugs stems from the FDA approval for use of the constituent nanomaterials such as liposomes, antibodies, chemotherapy drugs, particulate albumin, PEG, superparamagnetic iron oxide, and polylactic-co-glutamic acid, for clinical applications [138–141].

In addition to the above-mentioned types of nanomaterials being used for drug applications, use of CNTs as a substrate for development of novel diagnostic kits and therapeutic systems has caught the fancy of many pharmaceutical companies due to its unique pharmacokinetic properties despite the criticism that its high aspect ratio (length-to-width) could

make it comparable to asbestos [142, 143]. Although some recent studies suggest that inhalation of insoluble raw CNTs could be a source of inflammation, the fact remains that most of these studies were based on cytokine release, ROS elevations, complement activation, and cellular morphology changes when inhaled or added to cell cultures [144, 145]. This suggests that these *in vitro* observations need to be complemented with *in vivo* studies. It was found that CNTs can have toxic potential on human health [146–148], as was demonstrated in mice and rats. Ryman-Rasmussen et al. have demonstrated that when mice are allowed to inhale multiwalled CNTs (MWCNTs), platelet-derived growth factor (PDGF) overexpresses, inflammatory cell aggregates as well as MWCNTs, which are phagocytosed in the lung due to recruitment of macrophages [149]. Inhaled CNTs reach the subpleural tissue in mice to potentiate the development of subpleural fibrosis within 2–6 weeks of their bioaccumulation in the subpleural tissue [150]. Intraperitoneal injection of MWCNTs given to rats induced long-lasting inflammation and resulted in fibrous thickening and granuloma formation in the peritoneum in association with the induction of mesothelioma [151]. A similar experiment carried out in guinea pigs by Lam et al. at Peking University has shown that cytotoxicity of CNTs significantly increased upon increment of CNTs dose to alveolar macrophages. They found that SWCNTs also produced significant amounts of toxicity in comparison with MWCNTs at the same doses [146].

However, functionalization of CNTs can significantly reduce its toxicity [152]. We have also found that DNA coated SWCNTs show significantly low toxicity subjected to human umbilical vein endothelial cell lines (HUVECs) [153].

Moreover, the most standard assay being commonly used to determine toxicity of nanomaterials is the MTT assay, which is based upon the ability of the mitochondrial dehydrogenases to reduce the MTT salt resulting in the formation of a stable water insoluble MTT-formazan which is then extracted and photometrically quantified at 550 nm. There are many other ways to confirm toxicity level of the nanomaterials such as the WST assay, and the lactate dehydrogenase (LDH) assay. Worle-Knirsch et al. [154] demonstrated that determining toxicity level of CNTs *in vitro* only by the MTT assay is not conclusive and thus one should verify it with other different toxicity measurement protocols. They found that CNTs including SWCNTs react with some tetrazolium salts such as MTT but not with others, resulting in the formation of insoluble MTT-formazan which yield a fake cytotoxic effect. By contrast, detection with WST-1 reveals no cytotoxicity. In the study, they substantiated their results through the use of a variety of assays such as LDH, FACS-assisted mitochondrial membrane potential determination, and annexin-V/PI staining.

Many other particles under investigation for therapy and imaging such as quantum dots (Q-dots), gold-, iron-, or silica-based nanoparticles and nanoshells are under close observation *in vitro* for their associated toxicities [144, 155], including ROS activation, inflammation, and cytotoxicity [156]. It has so far been established that iron oxide particles are safe and widely used as imaging agents and sources of iron for treating anemia [157, 158]. Furthermore, Q-dots have been reported to be composed of metallic cores, especially from heavy metals such as cadmium, lead, or selenium that could significantly enhance its toxicity profiles [159, 160].

In the case of dendrimeric compounds, it has been observed that substantial alterations in charge and valency might induce significant changes in cell surface crosslinking, aggregation or activation and, therefore, causing possible increased toxic effects [161, 162]. Some dendrimer systems display very low toxicity levels – dendrimers carrying anionic groups being less toxic than those carrying cationic groups. Dendrimers also commonly show negligible or very low immunogenic response when injected or used topically. These properties make them highly suitable for drug delivery and biolabeling. In this regard, high biocompatibility is crucial both for preventing toxic reaction and for seeking biodegradability options. For example, large cationic PAMAM dendrimers induce platelet aggregation through disruption of membrane integrity [163]. PEGylation of dendrimers has been found to significantly reduce their toxicity and increase half-life in plasma [15, 164–166]. PEG-polyester dendritic hybrids were injected into mice intravenously (i.v.). Mice survived after 24 h, and no changes of organ pathology were observed in the liver, lungs, heart, kidney, or intestine [167]. PEGylated melamine dendrimer (G3, PEG 2000 termini) was administered at doses of 2.56 g/kg intraperitoneally (i.p.) and 1.28 g/kg i.v. into C3H mice, and all mice survived after 24 h and 48 h after i.p. and i.v. administration, respectively, with no liver or kidney toxicity noted [168].

One of the major concerns associated with the safety of liposomes such as N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP) lipid particles [169, 170] as well as synthetic agents such as PolyFect® and SuperFect® (Qiagen GmbH, Hilden, Germany) may be some deleterious side effects as well as off-target effects [171]. If these problems are resolved, liposomes might well realize their potential as a promising carrier system for future clinical use, particularly as part of a liver-targeted delivery system. Although PEGylated liposomes containing doxorubicin and amphotericin B, which have a high affinity for the liver, have been approved by the FDA [91, 172] for hepatic cancer therapeutic regimens, systemic administration of PEGylated liposomes does not seem to be a useful approach for the treatment of nonhepatic diseases, such as glomerulonephritis, at least when a passive transport approach is used [173].

4. Pharmacokinetics and pharmacodynamics

Pharmacokinetics (PK) is what the body does to a drug, and pharmacodynamics (PD) is what the drug does to the body. The therapeutic index of a drug refers to the ratio of therapeutic effect to toxicity. As different components will have different properties that affect the distribution, clearance, and metabolism of nanomaterials, it is indeed a huge challenge to understand the PK profile of any nanomaterials. The disposition of any drug usually is measured using four factors, namely absorption, distribution, excretion, and metabolism of

the drug. These factors determine the suitability of any novel nanoformulation with different cargos and require routinely regular iterative testing to determine the new PK behaviors. The developmental steps involved in the translation of a prodrug design to a clinically viable nanoformulation includes multiple steps of synthesis and purification along with a thorough physicochemical characterization before being evaluated for their PK, toxicity, and immunogenicity in the host. Indeed, it becomes challenging to predict the bioactivity of novel nanoformulations based upon available data for physicochemical description and PK profile in animals or human of others due to the existence of significant variability in the composition of their lattice structures.

Elimination of nanoparticles from the body has been particularly investigated. The hydrodynamic diameter and positive charge are known to be the key factors that in general are inversely related to glomerular filtration rate [174]. A common observation with the administered nanoparticles is that they accumulate in tissues/organs other than their intended sites of action such as targeted tumor. Such a substantial amount of nanoparticle accumulation in the tissues/organs may cause unwanted cytotoxicity and other side effects. Size and charge are two very key factors that decide the clearance of nanoparticles. Charge associated with nanoparticles causes adsorption of serum proteins that can affect their biodistribution, elicit immune response, and indiscriminately destabilize cell membranes and proteins. It is being observed that particle size with 3 nm in diameter or smaller gets stuck in the tissues nonspecifically; those 3–8 nm in diameter undergo renal clearance. Particle size of 30–80 nm in diameter are found to be sequestered in lung and leaky vasculature (e.g., tumor and inflamed tissue, via the enhanced permeation and retention effect), and particles larger than 80 nm become trapped by liver and spleen [175–177]. Moreover, dendrimers and polymers <8 nm in diameter primarily undergo renal clearance [178, 179].

Using the help of advanced imaging techniques such as magnetic resonance imaging, Kobayashi and Brechbiel [180] have demonstrated that varying the size or hydrophobicity of nontargeting gadolinium-labeled dendrimer constructs have significantly changed the excretion profiles of both kidney and liver. In another study, it has been shown that nontargeted, radiolabeled Q-dots modified with metal-ion chelates and a 600 Dalton PEG moiety are rapidly cleared from the blood to induce bioaccumulation in the liver within a few minutes [181, 182]. Typically, a size cut-off value of approximately 6 nm is monitored for filtration of globular proteins, although much larger biomolecules such as CNTs with high aspect ratios are easily filtered [183, 184]. Based on the physicochemical parameters as well as their lattice structure, nanoparticles such as Q-dots, with ligand numbers and sizes that permit renal clearance [185] upon conjugation with appropriate cargos, and ligands will allow interesting approaches to drug formulation. For example, the constitute agent may amass at the tumor site by the enhanced permeability and retention (EPR) effect, whereas its antibody ligand may ensure precision oriented binding to the tumor cell. The large particulate platform may accumulate in the liver before being excreted, whereas the released cargo might pass through via the kidney. Because of this type of complexity, studies on the PK of composite particles as well as the constituent cargos and metabolites are obvious routes to undertake so as to translate the concepts of novel nanopharmaceutical-based prodrug designs to clinically viable drugs on a spatial and temporal scale. Cargos can be formulated for triggered release under varying intracellular conditions of the target cell such as pH, redox, protease sensitivity, or esterase-sensitive linkers that degrade over time.

PEG is widely used and well accepted to enhance the PK of various nanomaterials. It reduces plasma protein adsorption and biofouling of nanoparticles that effectively reduces the renal clearance of relatively smaller drug molecules, thereby extending drug circulation half-life [66]. These studies have formed the rationale for initiating clinical trials of novel PEGylated composites such as NKTR-118 (PEG-naloxol) in Phase I for treating opioid-induced constipation, hepacid (PEG-arginine deaminase) in Phase II for hepatocellular carcinoma, and puricase (PEG-uricase) in Phase III for hyperuricemia. The PEGylated gold nanospheres, with long blood circulation times (in the range of 30 h), shows a remarkable propensity to accumulate in the liver and spleen of mice after as many as 7 days after injection, leading to acute hepatic inflammation and apoptosis [186]. Kaminskas et al. demonstrated in a rat model that 50% coverage of poly-L-lysine dendrimers by PEGylation can significantly improve plasma circulation as well as its biodistribution [187]. Later, this group conducted a different study with PEGylated (3) H-labeled poly-L-lysine dendrimers and found that after i.v. administration to rats this new composition offers considerably more resistance to biodegradation than the underivatized poly-L-lysine dendrimer cores [165].

Based on the patterns of its distribution and blood clearance kinetics, functionalized SWCNTs [184] and MWCNTs [188] upon being i.v. administered were found to have rapid renal clearance and translocation across the glomerular filter [189]. Even though the above-mentioned observations have stimulated much excitement about the possibilities of using CNTs as nanopharmaceuticals, skepticism arises from other studies showing contradictory data, with hepatic bioaccumulation along with slow hepatobiliary excretion when CNTs are modified with polymeric molecules [190] or with different surface chemistries [191]. Recently, observations made by Ruggiero et al. [192] strengthened the case for CNTs being projected as novel pharmaceutical adducts because of unique pharmacological behavior. They demonstrated that no active transport mechanism is responsible for this overwhelming glomerular translocation and that monostructured SWCNTs follow rapid elimination of most of the injected dose from the body within a timeframe of a few minutes. Only a small fraction (~15% of injected dose) is reabsorbed into the tubules of the kidneys and presumably, although not shown experimentally, recycled into the bloodstream, leading to slower, second-phase, excretion rates.

Silica is emerging as a promising material for the development of nanovectors possessing good biocompatibility. A huge amount of research is being done to restrict its

unspecified localization inside the body. Burns et al. reported a new generation of near-infrared fluorescent core-shell silica-based nanoparticles of hydrodynamic diameters of 3.3 and 6.0 nm with vastly improved photophysical characteristics. A neutral organic envelop over silica nanoparticle ensures complete prevention of adsorption of serum proteins facilitating efficient urinary excretion [174]. Kumar et al. recently demonstrated the biodistribution and clearance potential of 20–25 nm multimodal organically modified silica nanoparticles in nontumored nude mice [193]. In a tumor xenograft model, Lu et al. found that the accumulation of fluorescent labeled mesoporous silica nanoparticles with or without targeting molecule in the tumor is very quick and dense [194]. Souris et al. demonstrated that based on charge profile mesoporous silica either becomes quickly excreted from the liver into the gastrointestinal tract or remains sequestered within the liver [195].

5. Pharmacogenetics

Recent advances in the field of pharmaceutical sciences in combination with genomics have lead to the development of a novel branch of medicine catering to the specific needs of individual patients. Personalized medicine involves targeting, prediction, detection, diagnosis, prognosis, and treatment of disease using molecular biomarkers including but not limited to gene polymorphisms, RNA expression, proteins, metabolites, lipids, and others [196]. This therapeutic approach is based upon the information derived from patients using molecular tools obtained interdisciplinary branches of sciences such as pharmacogenetics, pharmacogenomics as well as pharmacoproteomics.

Pharmacogenetics is essentially the study which examines the role of genetics for an individual's response to drug treatment. Pharmacogenetics links the genetic differences (variation) in drug PK to the therapeutic response. By contrast, pharmacogenomics refers to study of the multiplicity of genes that ultimately determine drug behavior. Pharmacogenomics is in essence the whole genome application of pharmacogenetics, correlating gene expression or single nucleotide polymorphisms (SNPs) with drug efficacy and toxicity.

The most relevant pharmacogenetic targets as defined by the American Association of Clinical Chemists (AACC) encompass the cytochrome P450 enzymes CYP2D6, CYP2C9, CYP2C19, CYP3A5, CYP2B6 and thiopurine S-methyltransferase(TPMT), N-acetyltransferase 2 (NAT2), UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), multidrug resistance 1 (*MDR1*) gene and methylenetetrahydrofolate reductase (MTHFR) [197].

The genetic variations involved in the response of an organism to delivered drugs are indeed very complex. This interaction between genes and drugs can also modulate the pharmacotherapeutic outcome through either genetic variation or drug-regulated gene expression. This effect leads to phenotypic variation in pharmacotherapy (e.g., to differential pharmacological response) [198]. A detailed analysis of the molecular actions of drugs has clearly revealed that they put forth their effects via specific "molecular networks" involving several genes and proteins [199]. Recent studies demonstrated that epigenetic phenomena such as DNA methylation, histone methylation, and acetylation, as well as miRNA and RNA interference (RNAi) mechanisms, also significantly contribute towards establishment of specific gene expression patterns under divergent realms of both normal physiology and disease pathophysiology [200–202]. This finding means that both genetic and epigenetic factors must be considered in the efficient clinical translation of pharmacogenomics data to enhance their clinical validity and utility.

Nanobiotechnology along with other technologies enable the analysis of these complex multifactorial situations to obtain individual genotypic and gene expression information. It smoothens the progress of our understanding of disease mechanism, which is an integral component of personalized medicine along with pharmacogenomics, thereby contributing to drug discovery and development. Nanopore sequencing is an ultrarapid method of sequencing based on pore nanoengineering and assembly used for the detection of SNPs for gene diagnosis of pathogens [203]. In the area of pharmacogenetic diagnostics, gold nanoparticle based probe technology is used to build-up cutting-edge, clinical tests that facilitate rapid, multitarget detection of SNPs and similar DNA sequence variations that alter an individual's metabolism of specific drugs [204].

6. Overcoming chemoresistance

One of the major barriers in cancer chemotherapeutics is the acquirement of multidrug resistance (MDR) by recalcitrant cancer cells. In this situation, resistance to chemically unmodified drugs occurs due to active transport of these drugs out of the cell. Overexpression of the ATP-binding cassette (ABC) transporters, mainly P-glycoprotein (P-gp) [205], multidrug resistance-associated proteins (MRPs) [206], and breast cancer resistance proteins (BCRPs) [207], is one of the primary mechanisms of MDR. Once these transporters attach a substrate in the inner membrane, the substrate is then expelled into the extracellular space, thereby significantly reducing the intracellular levels of cytotoxic drugs below lethal thresholds and hence making the drug ineffective. Conjugation of cytotoxic agents with polymers alters the path of drug uptake from diffusion to endocytosis, thus reducing drug interaction with MDR transporters, resulting in increased intracellular accumulation and enhanced efficacy of the drug in resistant cells [208, 209].

Several studies have addressed the issue of MDR in sensitive and resistant ovarian carcinoma cells (A2780). Moderate increased uptake of the HPMA-copolymer doxorubicin (adriamycin, Adr) conjugate is achieved in Adr-resistant ovarian carcinoma cells (A2780/AD) in comparison to the free drug. Moreover, HPMA-Adr copolymer did not stimulate MDR in A2780 cells after repeated exposure, as confirmed by low expression levels of the *MDR1* gene. This data confirms that the conjugate is able to conquer the ATP-driven P-gp efflux pump [208, 209].

Gemcitabine (2′,2′-difluorodeoxycytidine; Gem) has become the standard treatment for locally advanced and metastatic pancreatic cancers [210, 211]. Gem in combination with other cytotoxic agents is also highly effective in the management of solid tumors such as ovarian, non-small cell lung, and pancreatic cancers [212, 213]. The major barrier of systemic Gem chemotherapy is due to its inefficient cellular uptake, metabolism as well as contributing to resistance induction, or both. A primary mechanism of resistance is due to the poor phosphorylation of Gem prodrug to its active triphosphate metabolites because of altered activation pathways. Several studies have indicated that the deficiency of deoxycytidine kinase (dCK), the key enzyme of the first phosphorylation cascade, is the most frequently explained mechanism of resistance to Gem [214]. Chemoresistance to Gem has also been assigned to the overexpression of ribonucleotide reductase (RRM2) and thymidylate synthase (TYMS) both involved in nucleotides synthesis [215, 216]. To address this issue, Couvreur et al. formulated a new squalenoyl nanoformulation of Gem [4-(N)-tris-nor-squalenoyl-gemcitabine (SQ-Gem)] [217, 218]. Recently, Réjiba et al. established that SQ-Gem does not significantly alter the expression of dCK, RRM2, and TYMS genes resulting in increased efficacy of Gem as a chemotherapeutic agent [219]. The antibody of P-gp functionalized water-soluble SWCNTs loaded with doxorubicin was found to escape the MDR of K562 human leukemia cells [220].

In a similar context, tri-block copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), (PEO-b-PPO-b-PEO), also known as pluronics or poloxamers, have appeared as promising candidates to modulate P-gp activity. These pluronics have a broad spectrum of activities. They restrain the P-gp drug efflux pump [221], which engages interaction of pluronic molecules with MDR cell membranes, thereby resulting in membrane microviscosity as well as inhibition of P-gp ATPase activity [222, 223]. Apart from this, they also reduce respiratory chain complexes in mitochondria of MDR cells and thus reduce ATP that deprives the MDR cells of the energy source [223]. Moreover, they help the production of ROS and simultaneously hinder the glutathione/glutathione S-transferase (GSH/GST) detoxification by decreasing GSH and inhibiting GST activity [222]. This is most noticeably seen in ATP depletion by pluronics, which associates with the expression level of P-gp in cancer cells [224]. One formulation containing doxorubicin and a mixture of pluronics L61, F127, and SP1049C has successfully completed Phase II human trials for the treatment of advanced esophageal adenocarcinoma [221].

7. Future direction

As we move into the 21st century, data from epidemiological studies from all over the world pertaining to the management of highly morbid and mortality causing diseases such as cancer suggest the existence of variability in terms of genetic polymorphisms among humans, which in turn, significantly contribute to polymorphisms in phenotypes. It is now well accepted that the variances in the phenotype are influenced by both genetic and environmental components. The existence of such variances is the *prima facie* cause for the failure of established drug regimens in the management of diseases such as cancer. This scenario has prompted the need to develop a comprehensive strategy catering to the needs of not only a population of a specific race but also to an individual.

Individualized medicine seems to be one of the major players in the development of effective therapeutic management against degenerative diseases such as cancer, cardiovascular diseases, diabetes, and AIDS. Recent advances in divergent disciplines of sciences such as nanotechnology seems particularly promising to form a new platform for facilitating the diagnosis and management of cancer (theranostic nanoparticles). A classical example of nano-based drugs pertains to the use of divergent classes/types of nanovectors such as liposomes, micelles, dendrimers which facilitate the site specific delivery of cargos such as siRNAs, proteins, genes, and combinatorial associations of drugs to their respective subcellular compartments such as nucleus and mitochondria. Such a site specific delivery of cargos can putatively form the rationale of a novel therapeutic regimen for treatment of not only highly morbidity causing diseases such as cancer but also other chronically debilitating diseases associated with the malfunctioning of the subcellular organelles such as mitochondria, which is emerging as an attractive therapeutic target.

One of the emergent concerns with the development of novel nano-based pharmaceutical drugs pertains to its stability, pharmacokinetic as well as its pharmacodynamic properties inside the human body/murine animal models. Research carried out extensively in this regard suggests that appropriate selection of nanocarriers based upon the size exclusion principle would significantly overcome the limitations of its bioaccumulation in localized regions of the human/murine body where it significantly modulates the outcome of the drug formulations being used as cargos. A major development in this regard has been the functionalizing of novel nanoplatforms such as SWCNTs and dendrimers which has contributed enormously to our understanding of its impact on keystone signaling paradigms responsible for extant variances found in hosts with the same therapeutic regimen.

Apart from this, use of multiplexed nanoparticles are being used extensively towards development of both *in vitro* or *ex vitro* diagnostics (e.g., tissue specimens and circulating tumor cells) to study cancer behavior and treatment response so as to provide a comprehensive strategy for individualized therapy. One of the novel applications of nanotechnology involves the use of biocompatible nanoparticles to overcome nonspecific organ uptake and ROS scavenging [225, 226].

Nanotechnology is also envisaged to provide a novel platform for imaging agents capable of penetrating solid tumors through the use of active pumping mechanisms, such as caveolin transcytosis and receptor-mediated endocytosis, or cell-based strategies, such as nanoparticle-loaded macrophages with minimal side effects.

Technologies that are driving the development of personalized medicine are derived from the concepts of pharmacogenetics and pharmogenomics along with their downstream processes to develop novel diagnostic kits as well as

therapeutic regimens in the management of highly morbid and mortality causing diseases such as cancer, diabetes, hypertension, along with other viral oncoprotein driven diseases (e.g., AIDS). Advances in nanomedicine will parallel that of personalized medicine and the interaction of both will justify the term personalized nanomedicine.

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Mayo Clinic profile

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Research focus

Angiogenesis: Dr. Mukhopadhyay's laboratory is focused on angiogenesis, tumor microenvironment, and vascular biology as it relates to cancer, diabetes, and other diseases. They are examining how tumors develop and, particularly, how they induce the angiogenic response that is essential for their survival. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) has been implicated in the new vessel development found in most tumors including renal cell carcinoma, pancreatic cancer, and breast cancer. Although the mechanism of these complex processes remain unclear, the laboratory is investigating the importance of VPF/VEGF as well as other angiogenic factors to elucidate the mechanisms by which VPF/VEGF functions in a variety of tumor models. Moreover, they are also studying the role of other angiogenic related factors such as insulin like growth factor (IGF-1), epidermal growth factor (EGF) in tumor angiogenesis and metastasis.

Vascular biology: A major limiting factor in the development of rational anti- or pro-angiogenesis therapy is our incomplete understanding of the basic steps and molecular mechanisms by which VEGF-A signals endothelial cell proliferation, migration, etc., through its receptors, VEGFR-1, VEGFR-2, and neuropilins. We are also investigating the crosstalk between dopamine receptors and VEGFRs that can influence both pathophysiological angiogenesis and edema. The long-term goal of this project is to elucidate the distinct signaling pathways mediated by VEGFRs in endothelial cells and its role on vascular biology.

Translational research for cancer therapeutics: Pancreatic cancer is often diagnosed late in its course when the cancer is already inoperable. Conventional treatments such as radiation and chemotherapy have proved largely ineffective. Our project will hopefully lead to the development of a rational therapy for pancreatic cancer that inhibits both tumor growth and angiogenesis. A potential drug development against pancreatic cancer by targeting IGF-1R/EGFR crosstalk and expression is the main aim of this project. Additionally, these drug development strategies will generate a new set of inhibitory molecules that can be applicable to other cancer types in which IGF-1R, EGFR, and angiogenic factors play important roles. Therefore, the knowledge gathered, as well as the new reagents that are in development, will have a usefulness extending far beyond pancreatic cancer.

Nanomedicine: The use of nanotechnology in biology has grown over recent years, incorporating the use of reagents such as nanoparticles to directly deliver bioconjugates. We are examining the use of these gold nanoparticles and other bioconjugates as messengers to deliver reagents that are capable of manipulating the angiogenic response *in vivo*. Our research will enable development of new nanomaterials that can be utilized in different aspects of medicine, including therapeutic treatment routes for translation into the clinical setting.

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Institutional focus

The Mayo Clinic conducts research to discover new treatments and improve patient care. Clinical practice observations become the basis for research studies, and the findings from research flow back into the practice to improve patient care and outcomes. It is an unbroken circle – with physicians, physician-researchers and career scientists working as teams to change the future of medicine.