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Nanotechnology for Cancer Diagnostics and Therapy – An Update on Novel Molecular Players

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Abstract: Nanotechnology has emerged as a "disruptive technology" that may provide researchers with new and innovative ways to diagnose, treat and monitor cancer. In fact, nanomedicine approaches have delivered several strategies, such as new imaging agents, real-time assessments of therapeutic and surgical efficacy, multifunctional, targeted devices capable of bypassing biological barriers to target and silence specific pathways in tumours. Of particular interest, has been the increased capability to deliver multiple therapeutic agents directly to bulk cancer cells and cancer stem cells that play a critical role in cancer growth and metastasis. These multifunctional targeted nanoconjugates are also capable of avoiding cancer resistance and monitor predictive molecular changes that open the path for preventive action against pre-cancerous cells, minimizing costs and incidence of relapses. A myriad of nanoconjugates with effective silencing and site-targeting moieties can be developed by incorporating a diverse selection of targeting, diagnostic, and therapeutic components. A discussion of the integrative effort of nanotechnology systems with recent developments of biomolecular interactions in cancer progression is clearly required. Here, we will update the state of the art related to the development and applications of nanoscale platforms and novel biomolecular players in cancer diagnosis, imaging and treatment.

Keywords: Nanotechnology, Cancer, Cancer therapy, imaging, molecular diagnostics, Nanoparticles, Nanosensors, nanoimmunochemotherapy, SERS.

INTRODUCTION

Cancer is singled out as the biggest cause of death in the world, predicted to reach 13.1 million cancer-related deaths by the year 2030 [1]. Cancer develops *via* a multistep carcinogenesis process entailing numerous cellular physiological systems making it a complex disease with hierarchies of cellular populations that demonstrate a range of differentiation phenotypes [2]. Current understanding of cancer development has shown that tumours comprise a complexity of cellular diversity that are responsible for the heterogeneous deleterious effect in organisms. For instance, in bulk tumours, the majority of cells may actually be non-tumorigenic end cells, where only a small subpopulation of cells within tumours is responsible for tumour initiation, growth, progression and recurrence. These "cancer stem cells" (CSCs) possess both self-renewal and differentiation capabilities, which mediate the high level of resistance against radiotherapy and chemotherapy [3-7]. In fact, CSC have been associated with increased resistance to chemotherapy agents due to insufficient elimination of these cells that will eventually lead to tumour recurrence [8]. Amongst the most frequent challenges of current cancer therapies are the nonspecific systemic distribution of antitumor agents, inadequate drug concentrations reaching the tumour site, intolerable cytotoxicity, limited ability to monitor therapeutic responses and in a high number of patients development of multiple drug resistance (MDR) and frequent relapse [9-12]. Also, targeted drug delivery will help eliminate the need for invasive surgery and radiation therapy, while more sensitive imaging strategies will allow for earlier detection and better prognosis [11]. The success of cancer therapies are limited by the development of new vectorisation platforms that are capable of specifically target, silence and deliver effective loads of anticancer drugs and molecules while lessening the impact of adverse side effects on normal tissues and organs. The development of combined therapeutic approaches capable of simultaneously improve the therapeutic index, reduce toxicity and deliver labile molecules specifically to the cancer cells is of utmost importance.

One of the most critical points in cancer treatment is early stage diagnosis, before tumour cells gain invasive capability and metastasise. Nevertheless, early stage diagnosis remains a significant challenge and delay in diagnosis contributes to the poor living quality of patients and low survival rates [13]. To date, cancer detection has been performed on the basis of clinical and pathologic staging (using conventional radiological and histopathological examinations) [6]. Priority needs to be given to innovative approaches that could help to identify biomarkers of early disease, delineate tumour margins, identify residual tumour cells and micrometastases, and determine whether a tumour has been completely removed [9-12]. Despite progresses in early cancer detection and diagnostics, many cancer screening techniques are still not very effective and characterisation of early cancer detection is urgently needed [14].

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Nanotechnology and nanomaterials in particular, are expected to provide a range of devices to treat cancer as their sizes are well matched to those of biologic molecules and structures found inside living cells [15]. Cancer nanotechnology offers a wealth of safety and innovative tools to treat and diagnose cancer, such as multifunctional, targeted devices capable of bypassing crucial biological barriers and to deliver multiple therapeutic agents directly to cancer cells and adjacent tissues around tumour microenvironment [16]. The development of nanoscale devices and structures has provided major breakthroughs in monitoring and fighting cancer [17-19].

Perhaps some of the most exciting advances in nanomedicine are multifunctional nanoparticulate systems for simultaneous imaging of tumour mass and drug delivery, tackling multiple biological targets associated with cancer development and/or progression. Nanosized vehicles are good drug delivery systems due to their biocompatibility, capability to accumulate *via* EPR effect, promoting less toxicity and side effects when compared to drugs alone. These cancer nanotheranostics systems combine the use of diagnostics (*e.g*. imaging) with therapy (delivery of anticancer drug and/or molecular actuators, such as gene silencing moieties) that can be delivered specifically to cancer cells (targeting). These nanoscale strategies can be engineered from components that (1) recognise disease at the cellular level, (2) are visible on imaging studies, and (3) deliver therapeutic compounds. Because of their small size, nanoscale devices that readily interact with biomolecules on both the surface and inside cancer cells can significantly improve the effectiveness and specificity of therapy. For example, specific targeting moieties can be used to direct specific agents towards CSCs, thus decreasing the chances of developing resistances leading to relapse [8, 11, 12, 20].

Here, we will provide an update to recent progress in nanoparticle (NPs) application to anticancer drug delivery.

NANOPARTICLES FOR EARLY CANCER DIAGNOSIS

Biomarker is defined by the National Cancer Institute (NCI) as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition" [21, 22]. There are several types of molecular biomarkers: DNA (*e.g*. a specific mutation, translocation, amplification, loss of heterozygosity), RNA, or protein (*e.g.* hormone, antibody, oncogene, or tumour suppressor). Cancer biomarkers allow the early detection of cancer, accurate staging, determining the response of cancer to chemotherapy agents, and for monitoring disease progression [23-26]. Potential biomarkers in cancer cells include stem cell-like markers, growth factors and their cognate receptors (*e.g.* EGFR and HER2), regulators of altered metabolism (PI3K/Akt/molecular target of rabamycin) and drug resistance (ABCB1, ABCG2 and MRP1). Moreover, different pluripotency-associated transcription factors (*e.g.* Sox2, Myc) and microRNAs, involved in the epigenetic reprogramming and acquisition of stem cell-like properties by cancer cells during cancer progression, may also be exploited as cancer molecular biomarkers to predict the risk of metastases, systemic treatment resistance and disease relapse of cancer patients [27]. It is now apparent that panels of cancer biomarkers, as opposed to the use of a single biomarker, will be necessary for reliable cancer detection and monitoring [25, 28, 29] – see (Table **1**). For additional information, please see [22, 27] and references therein.

Table 1. Common Biomarkers used in Cancer Detection [22]

Type of Cancer	Biomarker
Breast	BRCA1, BRCA2, CA 15-3, CA 125, CA 27.29, CEA, NY-BR-1, ING-1, HER2, ER/PR
Colon	CEA, EGF, p53
Oesophageal	SCC
Lung	CEA, CA 19-9, SCC, NSE, NY-ESO-1
Liver	AFP, CEA
Melanoma	Tyrosinase, NY-ESO-1
Ovarian	CA 125, HCG, p53, CEA, CA 549, CASA, CA 19-9, CA 15-3, MCA, MOV-1, TAG72
Prostate	PSA

Also, microRNAs (miRNAs), a group of small non-coding RNAs (approximately 22 nucleotides), that regulate the expression of their target genes by degrading target mRNA transcripts or inhibiting target mRNA translation, are being considered as a novel type of biomarkers that may play a role in accurate and early diagnosis, and also as prognostic determinants [30, 31]. Distinct miRNA expression patterns are associated with various cancers and anticancer drug resistance [30-32]. For instance miR-21 is overexpressed in many cancers, and its overexpression is significantly correlated with drug resistance in breast cancer [33-35].

Recently, the findings that human blood contains stably expressed miRNAs have revealed a great potential of circulating miRNA signatures as disease fingerprints to predict survival [36]. Due to their size, abundance, tissue specificity, and relative stability in circulation, miRNAs hold promise as unique accessible biomarkers to detect and monitor cancer [37]. For a more detailed state of the art in circulating miRNAs as cancer biomarkers see references [37, 38]. Additionally, the molecular characterisation of gene expression profiles have demonstrated a wide heterogeneity in cancer stem/progenitor cells, in primary tumours, exosomes, circulating tumour cells (CTCs) and disseminated cancer cells at distant metastatic sites. Therefore, technologies capable of detecting circulating tumour cells, circulating endothelial cells, circulating cancer stem cells and exosomes, which contain important miRNA signatures, directly from biological fluids may facilitate early diagnosis and treatment of metastatic cancers. The different gene expression profiles may contribute to improve the accuracy of diagnosis and predict the therapeutic responses and overall survival of cancer patients [27, 39]. Unfortunately, in early stages of cancer, tumour biomarkers are present in very low concentrations to be efficiently detected and even in a disease setting; circulating abnormal cells are rare events that are easily obscured by the overwhelming background material in whole blood [39].

The ability to measure panels of specific and selective cancer biomarkers directly at point-of-care (*e.g.* physicians' surgeries and clinics) may revolutionise cancer detection, monitoring and therapy. Nanomaterials and nanotechnology combined with modern instrumentation have shown the potential to measure multiple cancer biomarkers simultaneously [22, 39-41]. The use of multiplex labels in diagnostics and detection is only feasible due to a unique combination of chemical and physical properties of nanomaterials that allow biological molecules to be detected even at low concentrations [42]. Emerging inorganic nanomaterials, such as carbon nanotubes, quantum dots (QD), mesoporous silica/gold/superparamagnetic NPs, have been widely used in biomedical research with great optimism for cancer diagnosis and therapy, due to their unique optical, electrical, magnetic and/or electrochemical properties [2, 42].

Particularly, gold NPs (AuNPs) have been used for more than 2 decades for diagnosis, imaging and targeted therapy. AuNPs are of interest due to their unique intrinsic size-dependent properties, such as surface energy and light absorption or scattering, which are attributed to surface plasmon resonance (SPR) and surface-enhanced Raman scattering (SERS) [40, 41, 43, 44].

Probably, the more striking advances in biomolecular detection and characterisation of interaction with the physiological milieu have been provided by nanoparticle based SERS. AuNPs with SERS signatures are being used extensively in biomedical applications due to their inert biocompatible properties and high sensitivity in imaging application [45, 46]. SERS provides the capability of single molecule detection [47] and owing to the high sensitivity, inherent molecular specificity, and narrow bandwidth, SERS is an excellent diagnostic tool for cancer [48]. *In vitro*, SERS has been used for the identification of specific DNA sequences and mutations [49] and ultrasensitive detection of proteins and RNA [50]. Moreover, immunoassays employing SERS labels have been established to selectively target and quantify biomarkers to facilitate early diagnosis *in vitro* [51-54]. Many novel molecular diagnostics platforms using cheap single paper devices have been proposed [55, 56], which has been recently expanded to SERS. SERS can also be used on a paper platform for antibody–antigen detection using AuNPs functionalised to generate a prices fingerprinting of the interacting biomolecules [57]. This idea has been recently expanded to a highly sensitive non-invasive and rapid cancer screening platform encompassing exfoliative cytology and paper-based SERS technology consisting of plasmonic gold nanorods adsorbed on a piece of filter paper forming a flexible and three-dimensional heterogeneous scaffold [58]. Different and reproducible SERS spectra are obtained from normal and cancerous cells due to specific biomolecular changes in cancerous cells [58].

NANOPARTICLES AS IMAGING SYSTEMS

As discussed above an important goal in clinical diagnostics is the non-invasive detection of biological markers in diseased tissue to determine and visualise pathologic changes at an early stage, plan the therapy to be given, and further on track the response to therapy. Non-invasive imaging modalities, such as single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), optical fluorescence, or targeted ultrasound, are important tools in clinical diagnosis [59-61]. They are widely used for monitoring the disease status and for real-time evaluation of treatment response. The design of targeting and imaging agents that allow early detection of cellular abnormalities is crucial to make pathologic changes visible, quantifiable, and traceable over time. For the efficient delivery of both targeting and imaging labels, a variety of nanocarrier systems has been investigated [62, 63]. In particular, there is increase interest in the development of NPs systems for improved cancer imaging and diagnosis by MRI [64-66]. Still, of extremely high impact in the field of nanotheranostics has been the multiplexing ability of molecular imaging with encoded SERS nanoprobes that have also been utilised in living cells and animal tumour models [46, 67].

Nanotechnology has also promoted the possibility to multimodal imaging, which combines two or more distinct imaging modalities in a way that leverages the respective strengths and weaknesses of each imaging technology. Examples include combined PET-CT [67], PET-MR [68, 69] and MR-fluorescence imaging [70, 71], which often generates superior results compared to both modalities operating separately. The combination of positron emission tomography (PET) with X-ray computed tomography (CT) has become the gold standard in oncologic imaging [67, 72].

Magnetic nanoparticles (MNPs) have gained significant attention due to their intrinsic magnetic properties, which enable tracking through the radiology cornerstone, MRI [73]. This class of NPs include metallic, bimetallic, and superparamagnetic iron oxide NPs (SPIONs) [73, 74]. The latter of which has been widely favoured because of its inoffensive toxicity profile [75-78] and reactive surface that can be readily modified with biocompatible coatings [79-81] as well as targeting, imaging, and therapeutic molecules [80-83]. Currently, a number of SPIONs are in early clinical trials or experimental study stages [73, 74, 80], and several formulations have been approved for clinical use for medical imaging and therapeutic applications. Notable examples include: Lumiren® for bowel imaging [76], Feridex IV® for liver and spleen imaging [84], Combidex® for lymph node metastases imaging [85], and most recently, Ferumoxytol® for iron replacement therapy [86]. The physicochemical profiles of these SPIONs provide passive targeting, but not the higher level targeting offered by bioligands. Addition of bioactive molecules to the SPION surface can increase the targeting specificity of NPs [73, 74, 82, 87, 88], producing contrast agents that specifically illuminate targeted tissue and drug carriers that don not interact with healthy tissue [65, 73, 88-90].

MNPs can be additionally modified with other reporters to create multimodal imaging agents. For example NIR fluorophores have been attached to MNPs to create multimodal contrast agents that offer both the high spatial and temporal resolution and deep tissue penetration of MR imaging and rapid response and sensitivity of optical imaging [91]. Applications of these constructs include cell death monitoring, intra-operative imaging, and epithelial lesion detection [92]. The multimodal imaging approach can facilitate verification of the accuracy in tumour detection and provide additional information regarding the pathology of the tumour [65].

Molecular beacons are hairpin probes that have been used for RNA imaging in living cells [72]. The use of a single type of reporter dye on each molecular beacon allows multiple, optically distinct, molecular beacons to be visualised simultaneously (*i.e.*, multiplexing) [93]. This important attribute could potentially be taken advantage of to highlight the orchestration between various gene expression patterns in living cells. In fact, several groups have already demonstrated the feasibility of simultaneously imaging multiple genes in single living cells with molecular beacons [94-97]. Dual FRET molecular beacons have been used to detect K-Ras, surviving and oct4 mRNAs in HDF, Miapaca-2, and H1 cells, respectively [98, 99].

Due to their composition, liposomal nanoparticles (LNPs) form an excellent platform for the combination of imaging, diagnosis, and treatment of cancer. These spherical vesicles are composed of a bilayer of phospholipids with an aqueous interior and are able to accommodate lipophilic compounds within the lipid bilayer and hydrophilic compounds in the aqueous compartment [100, 101]. Coated with polymers (*e.g.*, polyethylene glycol [PEG]) to improve *in vivo* stability and taking advantage of the enhanced permeation and retention effect, liposomes have shown great potential in the field of nanosized drug delivery systems [102, 103].

Combination with appropriate targeting moieties, such as peptides or antibodies, may allow specific concentration of NPs in pathologic areas. Selection of targeting moieties specifically binding to diseased tissue while not affecting normal cells is essential for site-specific targeting [104]. A very promising target in this respect is to target tumour-induced angiogenesis. An example of such *in vivo* targets is αvβ3 integrin receptors, which are strongly overexpressed on the activated endothelium of angiogenic blood vessels [105]. Recently, Rangger and collaborators (2012) and Chen and collaborators (2004) have developed radiolabeled LNPs carrying a cyclic RGD peptide showing more favourable binding characteristics than linear or multimeric RGD peptides [106]. The number of targeting ligands on one nanosystem may be optimised to benefit from cooperative effect. Anderson and co-workers have used a highly echogenic decafluorobutane bubbles covalently coupled to a cRGD (cyclic Arg-Gly-Asp) peptide. The molecules of cRGD/bubble, exhibited a fivefold higher adhesion to immobilised integrins, relative to non-targeted bubble or a specific-targeted bubble [107].

The design of multifunctional molecular targeting and imaging probes is an attractive approach because many cancer types simultaneously express multiple receptor types as already described above [108]. Takara and collaborators described for the first time the *in vitro* and *in vivo* evaluation of multifunctional LNPs derivatised with two targeting sequences (RGD and SP). The LNPs were equipped with labels allowing *in vitro* and *in vivo* imaging of tumours as well as tumour-induced angiogenesis using multiple imaging modalities (SPECT, fluorescence microscopy, MRI). Dual-targeting with the RGD and SP peptide sequence on the same liposome (Hybrid-LP) was chosen because dual-targeting constructs have the potential to show synergistic effects *in vivo* and *in vitro* compared with their single-modified versions [109]. By using these two targeting sequences, it was possible to increase the specificity of the liposomes to reach their pathologic target and accumulate in sufficient amounts while preserving non diseased tissue. The elevated accumulation at the tumour site and the combination of LNPs with radioactive or imaging labels leaded to improved imaging qualities [109]. Multimodal liposomes (radiolabeled and paramagnetic) carrying a RGD-targeting sequence for SPECT/MRI have also been investigated [110]. Multifunctionality could lead to more personalised medicine by using LNPs equipped with a variety of drugs, imaging labels, and targeting structures.

In addition to liposomes, dendrimers and polymeric NPs have been considered one of the most promising nanomaterials capable to detect cancer and deliver multimodality therapy [2, 42]. Targeted delivery of specific NPs into the tumour can induce a local interaction with cancer cells and forces cancer cells to significantly increase the production of these biomarkers. Biomarkers detection becomes thus much easier and can provide an earlier diagnosis to doctors than biopsies. Early detections of cancers allow early and less burdensome treatments, increasing also the chances of recovery [2, 41, 42].

NANOPARTICLES FOR CANCER TARGETING – OVERCOMING MDR

Multi drug resistance (MDR) is a significant obstacle for the success of cancer treatment [111, 112]. Traditional chemotherapy or mono therapeutic strategies often fails to achieve expected results in cancer treatment due to MDR [113]. Mechanisms of MDR include decreased uptake of drugs, reduced intracellular drug concentration by activation of the efflux transporters such as P-glycoprotein (P-gp, encoded by ABCB1) and ABCG2 which are often overexpressed in cancer cells, modifications in cellular pathways by altering cell cycle checkpoints, increased metabolism of drugs, induced emergency response genes to impair apoptotic pathways and altered DNA repair mechanisms [114-116]. In fact, re-engineering nanoconjugates to circumvent drug resistance probably constitutes one of the main vectors of nanomedicine development in the near future.

Silencing of cancer-relevant genes is a challenging strategy to reduce resistance and to sensitise cancer cells towards chemotherapeutic agents [117]. Since the nineties, that several authors were able to modulate cancer MDR through inhibition of ABC transporter gene expression using ribozymes [118-121], deoxyribozymes [122] and antisense oligonucleotides [123]. Recently, small interfering RNAs (siRNAs), double-stranded RNA of between 21-25 nucleotides that selectively degrade mRNA blocking the production of a specific protein [124] have been applied *in vitro* for reversing MDR phenotype by targeting *ABCB1* or *ABCG2* mRNA [125-127]. A pioneering study using exogenous siRNA demonstrated the suppression of ABCB1 expression in conjunction with reversal of doxorubicin and paclitaxel resistance in human breast cancer cells [128]. MDR transporters also play a crucial role in protecting the CSCs from conventional chemotherapeutic agents [129]. CSCs subpopulations can be isolated based on cell surface marker profiles [130]. This characteristic may be used for specific targeting of CSCs and nanomedicine can offer a wealth of safety and innovative tools to specifically deliver multiple therapeutic agents directly to bulk cancer cells, CSCs and

agents directly to bulk cancer cells, CSCs and adjacent tissues around tumour microenvironment [8]. Indeed, the development of nanoscale devices and structures has provided major breakthroughs in monitoring and fighting cancer [15, 17-19].

In the last years different organic NPs, such as liposomes, lipids, micelles, and polymeric that co-delivery MDR-reversing agents and anticancer drugs (*e.g.* siRNAs and drugs like doxorubicin (DOX) and cisplatin (CIS) have been used to halt cancer progression, inhibiting the drug detoxification *via* suppression of cell defence mechanisms, activating apoptosis and DNA repair [111, 131, 132]. For instance, Xiong and collaborators used multifunctional micellar nanocarriers to co-deliver MDR-1 siRNA and DOX [133]**;** Sun and collaborators demonstrated that systemic administration of the micelle carrying polo-like kinase 1 (Plk1) specific siRNA and paclitaxel can induce a synergistic tumour suppression effect in the MDA-MB-435s xenograft murine model, requiring a thousand-fold less paclitaxel than needed for paclitaxel mono therapy delivered by the micelle and without activation of the innate immune response or generation of carrier-associated toxicity [134]; Ganesh and collaborators demonstrated that the combination of siRNA-mediated gene-silencing strategy (against mdr1, surviving, bcl-2) with chemotherapeutic agents (CIS) in CD44-targeting hyaluronic acid (HA)-based self-assembling nanosystems constitutes a valuable and safe approach for the treatment of MDR tumours [135]. Also, Zhou and collaborators targeted CSC persistence in chronic myeloid leukaemia (CML) using vectorised nanocarriers [136]. Resistance of CML CD34+ and primitive CD34+CD38− cells could be overcome using synthetic low-density lipoprotein (sLDL) particles. Although surface markers are partly shared with normal stem cells, there are still many differences, including signalling pathway and metabolic alterations in CSCs, which may be also exploited for selective targeted delivery of nanoscale drugs [8]. The molecular targeting of deregulated signalling pathways, Wnt/β-catenin, hedgehog, and Notch signalling, or other stem less markers which may contribute to the chemoresistance of cancer, have been also addressed recently [137-139].

Although organic NPs constitute the major strategy to deliver high amounts of drugs and MDR inhibitors, the use of inorganic NPs to reverse MDR in cancer has also been addressed. The most frequent systems usually combine silica, due to its higher drug loading capacity associated with high surface area to volume ratio and large pore volume [140-142]; magnetic [143-145]; or gold NPs, with an additional advantage of shape/size-dependent optoelectronic properties [146-147]. Other targeting moieties may be used against lymphatic vessels and block production of several growth factors that stimulate lymph-angiogenesis (*e.g.*, vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, platelet-derived growth factor subunit-B (PDGF-B), etc.) [150,151]. Also, tumour endothelial cells express several receptors, such as αVβ3 integrin, that stimulate intracellular signalling and gene expression involved in cell growth, migration, and survival of various types of tumour cells, making them also suitable targets [152]. These tumour markers have been successfully used *in vivo* to illustrate their potential as targeting moieties [19, 153, 154].

NPs based approaches hold great potential for cancer immunotherapy as a potent vaccine carrier. The use of NPs may allow the development of a broad armamentarium of targeted drugs against specific immune cells, which might overcome delivery mediated hurdles difficult to address with traditional approaches, such as small molecules or monoclonal antibodies [155]. Delivery of immunomodulatory agents across cell membranes *in vivo* has already been demonstrated as a desirable feature for selective delivery [156]. Nano-sized carriers hold great potential for advanced delivery systems for cancer immunotherapy, as such nanostructures can be used to more effectively manipulate or deliver immunologically active components to specific target sites to treat MDR positive cancers, as resistance to immunotherapy generally is unrelated to mechanisms of resistance to cytotoxic agents [156].

Nevertheless, there are still several issues to address before these approaches make an impression in the clinic: i) since the majority of drugs such as paclitaxel are hydrophobic and the MDR inhibitors like siRNAs anti-MDR associated genes are hydrophilic, this may impair the high loading capability in the same formulation; ii) how to achieve equilibrated molar ratios between molecules in loading multiple drugs in the same nanoformulation (often incomparable and difficult to control and quantify) [149, 157]; iii) possible interaction between chemotherapeutic agents and MDR inhibitors should be taken into account [158]; iv) toxicity of these novel nanoformulations should be addressed in a combined way [95]. Multifunctional nanoconjugates might be more efficient in treating MDR cancers by both targeting bulk cancer cells and CSCs, while simultaneously silencing genes responsible for MDR with low toxicity to normal cells [8, 159].

CONCLUSIONS AND FUTURE DIRECTIONS

Despite major advances in understanding cancer at the cellular and molecular levels and the underlying mechanisms involved in tumour survival and maintenance, clinicians still continue to face a daunting task of managing recurrent and metastatic cancers. Nanotechnology has been leading the way towards personalised oncology, in which cancer detection, diagnosis and therapy are tailored to each individual's tumour molecular profile. Also, nanotechnology platforms have boosted the efficacy of predictive oncology, where genetic and/or molecular biomarkers are used to predict disease development, progression and clinical outcomes.

The design and development of multifunctional nanosystems providing imaging (single or dual modality), therapy (a single drug or a combination of two or more drugs) and targeting (one or more ligands) built into/onto the same nano delivery system are intended to simultaneously perform diagnostics and therapeutics in real time - nanotheranostics. Such precision-guided multifunctional nanosystems portend to have tremendous utility for the better management of MDR tumours. Current knowledge regarding the safety of nanosystems, however, is insufficient and comprehensive acute and chronic toxicity *in vivo* studies should be carried out to identify the risks associated with NPs use. Translation of these nanosystems drug conjugates from the bench to the clinics has already been attempted but success is still far

from routine. Nevertheless, cancer nanotechnology holds promise to deliver a technological breakthrough for cancer patients.

CONFLICT OF INTEREST

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