NANOTECHNOLOGY AND CANCER

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Summary

Clinical medicine has seen tremendous improvements during past several decades. Despite its progress in many illnesses till date, cancer is not detected at the earliest subclinical stages. Patients still are exposed to highly toxic whole body chemotherapy. However with the discovery of nanotechnology which has emerged as a new tool for early diagnosis and as a targeted drug delivery system to the cancer cells will prevent mortality and increase the quality of life in cancer patients. Though extensive research is being done for various diseases like autoimmune, inflammatory and metabolic disorders, in this review we have stressed on the usage of various nanotechnological tools and methods in cancer detection and treatment. However like any novel technology, this also has some safety and ethical issues which have been discussed in this review.

Key words – Nanotechnology, cancer, diagnosis, treatment

1. Introduction

Nanometer (nm) is one billionth of a meter. At this size, substances will have unique physical and chemical properties that may be exploited for many biological uses. Nanotechnology which uses materials and devices at nanoscale (1-100nm) is considered as one of the greatest scientific innovations in today's scenario. Its uniqueness is that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications. One of the important areas of nanotechnology is "nanomedicine," which refers to highly specific medical intervention at the molecular scale for diagnosis, prevention and treatment of diseases (1).

Cancer, a condition caused by uncontrolled proliferation of cells is the second leading cause of death in western world. Despite various management options available today for cancer, no permanent cure has been found mainly due to *lack of diagnostic tools* in the detection of early stages of cancer and *toxic effects of the present treatment* modalities. Extensive research in the field of nanotechnology in last two decades has shown promise in answering these problems by helping in early detection, staging and treatment of cancer (2).

2. Nanotechnology in Cancer diagnosis –

Early diagnosis of cancer may result in successful outcome of cancer chemotherapy. But with the present investigational tools, the molecular transformation remains undetected until clinical manifestations have occured. This situation is mainly because of absence of highly sensitive techniques that can detect low level of tumor markers (3).
Nanotechnology may provide highly sensitive, less invasive and comfortable means of identifying and quantifying the markers which help in early cancer diagnosis and prognosis. This is possible due to the property of nanostructures to enter and analyze single cell. Hence they i) could detect molecular changes even at the earliest stages, ii) help the clinicians to conduct tests without physically altering the patient's cells or tissues and iii) reduce the tool size which makes screening faster and cost-efficient (2).

Nanotechnological tools used to *diagnose* the cancer include –
a) The bio barcode assay
b) Nanowires
c) Nanobiosensors
d) Cantilevers
e) Nanoparticles in MRI
f) Biochips
g) Gold nanoparticles
h) Quantum dots

a) The bio barcode assay **–** It is a sandwich immunoassay. In this assay, first magnetic microparticle surface is coated with monoclonal antibodies against target protein (DNA/ RNA) is mixed with serum containing target protein. Next gold nanoparticles coated with DNA barcodes are added to sandwich the target protein. Then magnetic field is applied which isolates specific barcodes (4-9). These DNA barcodes are identified using colorimetry. It is currently being evaluated as a potential tool to identify a minimal rise in prostate specific antigen (PSA) well before conventional tests. The protein markers for testicular cancer are also being tried using this assay (9).

b) Nanowires – Nanowires are miniscule devices with a diameter between 10 -20nm. They can detect cancer marker (protein) as low as one hundred billionth of total protein present in a drop of blood. These nanowires conducting a small current are modified by coating with cancer marker specific antibodies. When the proteins (markers) come in contact with their specific antibodies it sparks momentary change in conductance that gives indication of their presence. The detectors differentiate among various cancer markers both through the specific antibodies against them and the characteristic length of time before dislodging. In this way a nanowire array can test a mere pinprick of blood in just minutes (10).

c) Nanobiosensors – The combination of nanotechnology, biology and photonics opens the possibility of detecting the molecular changes inside the cancer cell. In nanobiosensor, the sensor is coated with cancer specific antibodies or other bio recognition ligands. Like in nanowires when target protein comes in contact with these antibodies there is production of electrical, optical or mechanical signal which are detected and analyzed. The sensors can operate in liquid and gas phase. They can detect binding events directly and there is no need for costly, complicated and time consuming labeling chemistries such as fluorescent dyes or the use of bulky and expensive optical detection systems (11).

d) Nanoscale cantilevers – These are microscopic, flexible beams resembling a row of diving boards that are built using semiconductor lithographic techniques and coated with antibodies or molecules capable of binding to the biomarkers of cancer. As a cancer cell secretes its molecular products, the antibodies coated on the cantilever selectively bind to these secreted proteins, changing the physical properties of the cantilever and signaling the presence of cancer. Thus nanoscale cantilevers, constructed as part of a larger diagnostic device, provide an alternative to PCR and can provide rapid and sensitive detection of cancer-related molecules like oncogenes. One such example is use of gold coated nano cantilever to detect activity of the gene 1-8U produced by melanoma cells (12).

e) Nanoparticles in Magnetic Resonance Imaging (MRI) – Conventional contrast agents used for imaging in MRI like, free manganese (Mn^{2+}) and Gadolinium (Gd^{3+}) are found to cause cardiovascular and CNS toxicity respectively. To overcome these, less toxic insoluble manganese oxide (MnO) nanoparticles (25nm) encapsulated in a biocompatible PEG phospholipid shell are being studied. They were tested by injecting into a mouse's tail, and subjecting it to an MRI scan 72 hours later. The images were as clear as those usually obtained from dissection and histological examination. By conjugating the MnO nanoparticles to a tumour-specific antibody, imaging of selective tumour cells is also possible (13).

Superparamagnetic iron oxide (SPIO) is also being studied to enhance the contrast images. Conjugation of folate and polyethylene glycol onto superparamagnetic nanoparticles results in increased uptake in cancer cells that may be imaged by MRI (14-15).

f) Biochip – Nanotechnology on a chip is a new paradigm for total chemical analysis system. A biochip, consists of a one-centimeter by one centimeter array that comprises anywhere between several dozen and several hundred "dots," or small drops. Each of these drops contains a unique protein, antibody that will attach to a particular tumor marker (DNA sequence or antigen). A tumor, even in its earliest asymptomatic phases, can slough off proteins that find their way into a patient's circulatory system can be detected and analyzed by this novel method (16).

g) Gold nanoparticles – Gold nanoparticles are known for their property of scattering and absorbing light. Conjugating gold nanoparticles with specific antibodies to cancer markers make cancer detection much easier. The best results have shown to come with particle size of 35nm. When seen under microscope, gold nanoparticle bound cancer cell shine in comparison with non cancer cells. These nanoparticles are affordable (as only microscope and white light is required), provide instantaneous results and can detect millions of different DNA sequences simultaneously (17).

h) Quantum dots – Quantum dots (QDs), tiny light-emitting particles on the nanometer scale that have attracted widespread interest in biology and medicine. QDs have got unique properties

like intense and stable fluorescence for a longer time and ability to absorb and emit light very efficiently, which make them ideal for detecting tumors in early stages. QDs have been covalently linked to various biomolecules such as antibodies, peptides, nucleic acids and other ligands for fluorescence probing applications. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals (18).

3. Nanotechnology in cancer therapy –

Nanotechnology can increase the number of highly effective therapeutic agents and possess potential to change cancer therapy for the better . This technology has proved to be very useful in cancer therapy allowing effective and targeted drug delivery by overcoming many biological, biophysical and biomedical barriers that the body stages against a standard intervention such as the administration of drugs or contrast agents without harming healthy, neighboring cells.

3.1. Types of nanoparticles for drug delivery –
Many kinds of nanoparticles like nanoscale liposomes, pegylated nanoparticles, gold nanoparticles are loaded with anticancer drugs in the management of cancer.

a) Nanoscale liposomes – One of the earliest applications of nanotechnology in medicine was the use of liposomes in nanoscale range to deliver chemotherapy to the tumour (3). Liposomes work by encapsulating therapeutic agents in a one or two-layer lipid-containing membrane. The liposomes remain in the circulation for prolonged period of time and accumulate passively in the tumours via an enhanced permeation and retention effect (EPR) (19). The EPR effect arises from the unique morphology of the tumour vasculature which is highly leaky with pore sizes as large as 600nm that allow the nanoscale liposomes to easily extravasate out into the tumour. This provides several potential advantages like, i) improving the distribution and safety of poorly soluble agents, ii) slowing the rate of release and clearance of the therapeutic agents, and iii) increasing the time of circulation to allow efflux at areas of abnormality, which often are associated with hyperpermeability of vasculature endothelium (20).

b) ***Stealth nanoparticles (PEG-coated liposomes)-*** Among the different polymers investigated in an attempt to improve the blood circulation time of liposomes, polyethylene glycol (PEG) has been widely used as polymeric steric stabilizer. Stealth liposomes are important in cancer treatment for their passive targeting effect, which may lead to preferential accumulation in tumor tissue, but this phenomenon is not fully understood. Stealth liposomes are able to lodge in the interstitial spaces among tumor cells. Once they enter tumor area, they accumulate in the extracellular fluid surrounding the tumor cell without entering it. The drugs are delivered from here into the cell for their action. Eg: doxorubicin or cisplatin (21).

c) Gold nanoparticles – Gold nanoparticles initially used for the detection of cancer in its early stages are now being tried for treatment of cancer. The ability to absorb light by gold particles was used to kill cancer cells. The study showed that malignant cells which are attached with nanoparticles require less than half the laser energy required for killing benign cells.

3.2. Modified nanotechnologies for treatment of cancer:

a) Non surface modified nanotechnology –

At the tissue level, upon intravenous injection, colloids are opsonized and rapidly cleared from the bloodstream by the normal reticuloendothelial system (RES) irrespective of particle composition (22-24). Thus the liver acts as a reservoir towards nanoparticles, liposomes etc. This biodistribution can be of benefit for the chemotherapeutic treatment of mononuclear phagocyte system (MPS) localized tumours.

b) Surface modified nanotechnology –

Although conventional liposomes are important development in drug delivery, their usefulness is limited by their rapid blood clearance and recognition by mononuclear phagocyte system (MPS). A major breakthrough in the liposome field consisted in the use of phospholipids substituted with polyethylene glycol (PEG) chains. These pegylated liposomes have circulating half lives of 45 hours as opposed to a few hours or even minutes for conventional liposomes.

A similar strategy has been applied to nanoparticles. PEG can be introduced in two ways – either by adsorption of surfactants or by the use of block or branched copolymers like polylactic acid (PLA) or polyalkycyanoacrylate.It is seen that not only the surface characteristics of the particles but also their size are the keys for the biological fate of these nanodevices because these parameters can prevent their uptake by MPS macrophages.

c) Addressed nanotechnologies for the treatment of cancer -

Specific antibodies or ligand targeting proteins expressed on cancer cell membrane or endothelial cells lining the newly generated blood vessels into the tumor are among the possible options to perform the active targeting of nanotechnologies toward tumoral sites. Examples include galactolipids that bind to the asialoglycoprotein receptor of the human hepatoma Hep G2 cells. In some cases, active targeting needs a receptor mediated cell internalization to occur (e.g. antibody coated liposomes). This approach has been proven to be more efficient because of the better accessibility of the antibody toward its corresponding antigen (25).

3.3. Anticancer drugs –

a) Doxorubicin –

In comparison to plain doxorubicin, superiority of *doxorubicin loaded nanoparticles* (targeted with the aid of biodegradable poly-alkylcyanoacrylate) has been demonstrated in a murine hepatic metastasis model. The reduction in the number of metastases was much greater with doxorubicin loaded nanoparticles, particularly if the treatment was given only when the metastases were well established. Additionally, with this type of doxorubicin loaded with nanoparticles, impressive results were obtained in cases of multidrug resistance hepatocellular carcinoma, which is one of the most prevalent cancers (26).

Doxorubicin hydrochloride encapsulated in stealth liposomes (DOXIL) that incorporates PEG, a molecule that allows further evasion of uptake and removal by reticuloendothelial system. This has been approved for AIDS related Kaposi's sarcoma, metastatic ovarian cancer, breast cancer, non Hodgkin's lymphoma, non small cell lung cancer, etc. Because of the long circulating

liposomes promotes extravasation of the drug, new toxicities may emerge like hand foot syndrome (27).

Thermosensitive liposomes containing doxorubicin in a hamster model of osteosarcoma were administered followed by application of heat to the primary site. This resulted in inhibition of tumour growth and metastasis (28).

Magnetic liposomes enveloping doxorubicin was used in osteosarcoma model. After intravenous injection a magnetic field applied over the affected limb brought about increased drug at the site, suppression of primary tumour and decreased lung metastasis (29).

Liposomal doxorubicin coupled with transferrin increases antitumour effect on C6 glioma cells and colon tumour cells because of ability of the transferrin receptors to be cell internalized with its specific ligand (30).

b) Paclitaxel –

Standard formulation of paclitaxel requires the use of solvents, such as Cremphor-EL, which contribute to some of the toxicities commonly associated with paclitaxel-based therapy. Nanoparticle albumin-bound paclitaxel (*Nab*-paclitaxel/ ABRAXANE) is a novel solvent-free formulation of paclitaxel. The formulation is prepared by high-pressure homogenization of paclitaxel in the presence of serum albumin into a nanoparticle colloidal suspension. The human albumin-stabilized paclitaxel particles have an average size of 130 nm. *Nab*-paclitaxel has several practical advantages over Cremphor-EL-paclitaxel, including a shorter infusion time (30 min) and no need of premedications for hypersensitivity reactions. The *nab*-paclitaxel formulation eliminates the impact of Cremphor-EL on paclitaxel pharmacokinetics and utilizes the endogenous albumin transport mechanisms to concentrate *nab*-paclitaxel within the tumor. A recent Phase III trial compared *nab*- and Cremphor-EL-paclitaxel in patients with metastatic breast cancer. Patients treated with *nab*-paclitaxel experienced a higher response, longer time to tumor progression and, in patients receiving second-line or greater therapy, a longer median survival. Patients treated with *nab*-paclitaxel had a significantly lower rate of severe neutropenia and a higher rate of sensory neuropathy. The preclinical and clinical data indicate that the *nab*-paclitaxel formulation has significant advantages over Cremphor-EL-paclitaxel (31).

c) Cisplatin –

Cisplatin is the most effective cytotoxic agent against many cancers. Its usage, however, is limited due to inefficient uptake by the target cells. A *nanoscale liposomal formulation* of cisplatin is reported to partly overcome this limitation. Physicochemical characteristics of the liposome-cisplatin preparation, including its size, stability, encapsulation efficiency, and cytoplasmic internalization efficiency, play a significant role in an effective usage of liposomal formulations. In comparison with liposomes without cisplatin which get internalized into the cell and do not induce cell toxicity, cisplatin-encapsulated liposomes of ~250 nm diameter (nanoliposomes) are most efficiently internalized and induce cell toxicity in a time-dependent manner (32).

3.4. Nanotechnology in gene therapy of cancer –

Gene therapy may be considered as a potential approach for cancer treatment either to transfect a tumour suppressor gene or to inhibit the expression of oncogenes or to induce an immune response. Most common genetic therapy strategy is transfer of genes responsible for the production of immune stimulants such as cytokines. Cationic lipids and liposomes are probably the most popular and ancient nanotechnology for gene delivery. They interact electrostatically with DNA allowing the condensation of this molecule and cell internalization likely through endocytosis. Dioleoyltrimethyl ammonium propane (DOTAP) is a cationic lipid used commonly for this purpose (33).

4. Nanoparticle Vaccines for cancer -

Vaccines represent a promising area in which nanotechnology is likely to have a high impact in the prevention and treatment of cancer. A cationic lipid (TFL2-3) formulation enclosing a plasmid vector encoding Epstein-Barr virus (EBV) oriP and EBV nuclear antigen with autologous tumor cells plus IL-12 and IL-18 genes in a melanoma mouse model resulted in tumor suppression associated with interferon production (34). Archaeosomes, vesicles of ether glycerolipid (*Methanobrevibacter smithii*) containing ovalbumin antigen as an immunostimulant in mouse models of melanoma cells (both positive and negative for ovalbumin antigen expression), showed decreased tumor growth in ova-expressing tumors which was believed mediated by cytotoxic T lymphocytes (35).

5. Safety issues –

Though many kinds of nanoparticles, loaded with a variety of drugs and imaging agents, are treading their way toward the clinic, the safety of them is an important concern. Two factors that could make nanoparticles a particularly serious occupational risk include their size and their massive surface area. Due to their ultra-small size, nanoparticles can penetrate cell membranes and integrate themselves into larger molecules. They can resist cellular defense systems but are large enough to interfere with cell processes. Two types of nanoparticles (silica and C60 fullerene), studied in MCF-7 and breast cancer cells have shown an increase in DNA damage (single and double-stranded breakages) with both dose and time-dependent results. Although DNA breakages do not necessarily mean a substance is cancer-causing, it is widely accepted that chemicals causing DNA damage are highly likely to promote mutations which can lead to cancer.

There are different types of nanoparticles including the fullerenes (carbon nanotubes) used as carriers for drugs are known to attract electrons and cause generation of damaging free radicals. Modifications to the carbon surface of nanotubes, making them more soluble, have been shown to reduce their toxicity.

As the majority of nanoparticles are intended to travel to tumors through the bloodstream, the effects of nanoparticles on blood cells are of particular concern to those developing nanoparticle-based therapeutic and imaging agents. Pegylated nanoliposomes have found to achieve

concentration in the blood stream that produced no untoward biological effects on blood cells. But higher levels of the test nanoparticles did slow blood clotting. So, the researchers noted that nanoparticle effects on blood cells are likely to depend on the particle size and surface properties (36).

Summary and Conclusion

Nanotechnology offers an important opportunity to study and interact with normal and cancer cells at the molecular and cellular scales, and during the earliest stages of the cancer process. This will bring enabling technologies for:

- Imaging agents and diagnostics that will allow clinicians to detect cancer in its earliest stages
- Multifunctional, targeted devices capable of bypassing biological barriers to deliver multiple therapeutic agents directly to cancer cells and those tissues in the microenvironment that play a critical role in the growth and metastasis of cancer
- Novel methods to manage the symptoms of cancer that adversely impact quality of life

So, there is no doubt that nanotechnology will continue to have a profound and positive impact and hold great promise for diagnosis and treatment of cancer. The ultimate goal of the nano-technology is rapid *bench-to-bedside translation* of the research to patients with cancer.

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