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Review Article

# Nanoparticle therapeutics: Technologies and methods for overcoming cancer

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ABSTRACT

It is anticipated that by 2030 approximately 13 million people will die of cancer. Common cancer therapy often fails due to the development of multidrug resistance (MDR), resulting in high morbidity and poor patient prognosis. Nanotechnology seeks to use drug delivery vehicles of 1–100 nm in diameter, made up of several different materials to deliver anti-cancer drugs selectively to cancer cells and potentially overcome MDR. Several technologies exist for manufacturing and functionalizing nanoparticles. When functionalized appropriately, nanoparticles have been shown to overcome several mechanisms of MDR *in vivo* and *in vitro*, reduce drug side effects and represent a promising new area of anti-cancer therapy. This review discusses the fundamental concepts of enhanced permeability and retention (EPR) effect and explores the mechanisms proposed to enhance preferential "retention" in the tumour. The overall objective of this review was to enhance our understanding in the design and development of therapeutic nanoparticles for treatment of cancer.



## 1. Introduction

Cancer is a disease that kills millions of people every year and it does not discriminate between age, gender, ethnicity or social economic state. Cancer may arise from external insults that damage DNA, such as environmental exposure to carcinogens i.e. UV light, cigarette smoke, by spontaneous genetic mutations or by epigenetic changes [1].

Cancer is commonly treated by chemotherapy, which uses cytotoxic agents that are not selective to cancerous cells and are therefore toxic to healthy labile cells. Chemotherapy often fails, even in an adjuvant setting after radiotherapy and surgery to remove the tumour [1,2]. One of the reasons cancer chemotherapy fails is because tumours are very resilient and develop resistance to several multimodal cytotoxic drugs; this is termed multidrug resistance. Understanding the molecular mechanisms of multidrug resistance is essential for the development of more suitable treatments that reduce morbidity, improve quality of life and improve patients' prognosis.

Over the last few years a significant number of novel cancer treatments have emerged, but not many continue to be under clinical investigation and some have proved unsuccessful at curing cancer. Nanotechnology however seems promising, as it aims to develop nanoparticles that can evade the immune system, and use the tumour's own environment to deliver cytotoxic drugs such as cisplatin, paclitaxel or doxorubicin selectively to cancer cells, avoiding efflux pumps. The aim of this literature review was to briefly explain the molecular aetiology of cancer and the development of multidrug resistance from the point of view of potential nanoparticle therapy approaches. It will also look into some of the technologies for manufacturing nanoparticles, discuss nanotoxicity and provide some insight into future research.

## 2. The Big Scary C Word: cancer

Cancer is the term given to describe the malignant, autonomous growth of cells that have undergone epigenetic changes and genetic mutations and thus escaped normal cell cycle and displayed various degrees of similarities to their precursors [1].

Cancer poses a huge burden for the health system; it is highly prevalent in both developed and underdeveloped countries. In 2012, cancer was the leading cause of death resulting in approximately 8.2 million deaths worldwide [1]. The global burden of cancer is expected to reach >13 million deaths per year by 2030 and the overall age-standardized cancer incidence rate is

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approximately 25% higher in men than in women [1]. In regions such as Australia and New Zealand the cancer incidence rate is 365 per 100,000 [2].

Cancer results from insults to DNA that cause genetic mutations, or from spontaneous genetic mutations that occur in somatic cells randomly, or through exposure to environmental carcinogens such as cigarettes, human papilloma virus (HPV), and human immunodeficiency virus (HIV). These account for over 90% of all cancers [3,4]. The remaining 10% have an inherited origin such as breast cancer 1 (BRCA1), breast cancer 2 (BRCA2) for familial breast cancer [5], or adenomatous polyposis coli (APC) for familial adenomatous polyposis colorectal cancers [4]. An understanding of the molecular basis of cancer is necessary in order to develop appropriate methods of treatment.

### 2.1. The genetic basis of cancer

Cancer originates from genetic mutations that inactivate tumour suppressor genes and/or activate oncogenes [1,5]. There are several types of mutations, and these include frameshift mutations (altered reading frame), missense mutations (altered amino acid), or nonsense mutations (truncation of protein product) [5,6]. Mutations may arise from a combination of environmental and life-style factors but it is difficult to establish a cause–effect relationship. These mutations often affect proteins responsible for the repair of DNA damage, cellular function, cell division and programmed cell death (apoptosis) such as p53; thus, loss of these functions is beneficial for cancer formation and progression [1,5,6]. In normal cells, following DNA damage, several mechanisms are in place to repair the damage, but if the damage cannot be fixed, the cells undergo apoptosis (without inflammation). This mechanism is classically lost in >50% of all cancers (ref). A classic tumour suppressor gene affected by mutation is p53; p53 mediates apoptosis when DNA repair fails, by binding to ubiquitin ligase and inducing proteasomal degradation; ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) protein recognizes DNA damage and phosphorylates p53 thus activating it [1]. Another example is RAS, an oncogene from the Ras-superfamily of proteins involved in the regulation of signal transduction pathways and growth regulation that involve GTPase, often activated by viruses such as HIV and HPV to induce tumorigenesis [5].

There are several genes involved and identified as either tumour suppressor or oncogenes, and they have several functions; however, there are several other genes that are also associated with malignant neoplasia that has not been classified as such but also can cause cancer. These fall into six main categories, or hallmarks of cancer [1,5,7,8] that represent classic characteristics of cancer not seen in healthy cells thus permitting intervention. These are as follows:

(1) Loss of contact inhibition via aberrant cell adhesion properties.
(2) Failure to undergo apoptosis (dysfunctional cell cycle checkpoints) or evading growth suppressors.
(3) Exaggerated response to growth inducing agents or reduced responsiveness to growth regulating signals.
(4) Immune defence unresponsiveness.
(5) Irregular or induced angiogenesis.
(6) Immortalization through the gain of telomerase activity.

### 2.2. The epigenetic basis of cancer

Several epigenetic mechanisms of malignant neoplasia have been elucidated recently, but for the purpose of this review they will only be briefly explained.

Epigenetic inheritance is the flow of information between cell generations that is not encoded in the primary sequence of the DNA, and is mediated partially by changes in histone acetylation, histone methylation and DNA methylation [6,7,9]. Transcription of genes is only possible if the appropriate transcription factors are present, if histones are appropriately acetylated and methylated and if cytosine residues in promoters are unmethylated [7].

Epigenetic factors that result in malignant neoplasia, cancer, include global DNA methylation resulting in the reduction in the proportion of methylated CpGs [9,10]. Hypomethylation of CpG islands activates oncogenes such as H-Ras and cyclin D2, and the multi-drug resistance gene MDR-1 [10]. Hypomethylation of pericentrometric satellite sequences causes the decondensation of mitotic recombination, aneuploidy and chromosomal instability as seen in many carcinomas [11]. Hypermethylation of regional DNA, consequently CpGs, is also carcinogenic. Hypermethylated CpG islands in the promoters of tumour suppressor genes (TSGs) cause silencing of genes resulting in one of two hits: (1) one allele of TSG may be mutant; and (2) hypermethylation selectively silences the wild-type allele [11,12]. Hypermethylation of TSGs may impact on several genes resulting in malignant neoplasia. For example, hypermethylation of *GSTP1* (encoding glutathione S-transferase pi) genes, which encodes for a family of enzymes that play an important role in detoxification, can cause accumulation of DNA adducts, hypermethylation of *CDKN2A* (encoding cyclin-dependent kinase inhibitor 2A) which encodes for proteins involved in tumour suppression, can cause cell cycle deregulation, and P14ARF (encoded by an alternate reading frame within the *CDKN2A* gene) can cause loss of p53 function, and *APC* (encoding tumour suppressor proteins) which causes gain of stem cell function [12].

### 2.3. Inflammatory basis of cancer

About 15–25% of cancers arise from chronic inflammation, sometimes associated with infection (i.e. Crohn's disease, hepatitis, cystitis and others) [13,14]. Inflammation on its own is not sufficient to cause carcinogenesis but can promote the right environment (rich in inflammatory cells, growth factors, DNA-damaging agents such as oxidative stress), and promote the proliferation of cancer cells [13,14].

In colorectal cancer, inflammation turns off the inhibitor of NFκB (nuclear factor kappa B) resulting in elevated release of pro-inflammatory cytokines, damaging the gastric mucosa [13]. Monocytes can be recruited to the site of inflammation but the immunosuppressive tumour environment alters the phenotype of these cells resulting in a scavenger phenotype that produces growth factors such as hepatocyte growth factor (HGF), epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF). VEGF in particular results in hypoxic activation of HIF-1α (hypoxia inducible factor 1) and soluble CSF-1 (colony stimulating factor 1) in avascular tumour areas which favours the tumour. Macrophage migration inhibitor factor (MIF) and suppressor of p53, as well as tumour growth factor β (TGFβ) which are immunosuppressive growth factors are also activated in an inflammatory environment [14,15]. These effects lead to the propagation of DNA damage which can further promote carcinogenesis.

## 3. Current methods of cancer treatment

The appropriate cancer therapy treatment is dependent on the type and stage of cancer, age and gender of the patient. The three main methods of treatment include chemotherapy, radiotherapy and surgery. Chemotherapy and radiotherapy are commonly used methods of cancer treatment, often given as adjuvant to each



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