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Nanotechnology May Replace Existing Treatments for Cancer

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In 2002, 23% of all deaths in the United States were caused by cancer making it the second biggest killer, only ranking behind heart disease (Jemal et al., 2005). Every year, over a half million Americans die of cancer and more than a million are diagnosed with the disease. It is also the second biggest killer among children, with nearly 12% of all childhood deaths coming from the disease (Jemal et al., 2005).

Cancer is a disease in which cells proliferate uncontrollably (Campbell et al., 2002). Unlike most cells, cancerous cells do not display density dependent growth, meaning they divide with little spatial regulation (Moossa et al., 1990). Moreover, these cells have the ability to spread by breaking into blood vessels and moving to other systems (Moossa et al., 1990).

Cancer can be fatal due to a combination of its properties. For instance, cancerous cells lose their ability to function normally. That is, they stop responding normally to cellular signals and therefore no longer perform their job (McKinnel et al., 1998). Not only do cancer cells cease working, they also affect neighboring cells because cell division and metabolism require nutrients and energy; eventually the cells require more nutrients than the body can provide and slowly organ systems begin to fail, a process known as cachexia (American Cancer Society, 2000). Additionally, the growths themselves can cause immense pain or death in hollow organs (such as the colon) by blocking the lumen and preventing proper function. Moreover, tumors can cause pressure on the brain which can lead to brain failure, seizures, or partial lack of function depending on the location of the tumor (McKinnel et al., 1998).

The formation of cancer requires several genes to be altered through mutations, which can be caused by spontaneous errors in replication or by exposure to carcinogens that alter nucleotides or break the DNA strand. In order for a mutation to lead to cancer, it has to perpetuate the cell cycle (Kruh et al., 2000).

The cell cycle is a highly regulated process that ultimately results in the division of one cell into two (Campbell et al., 2002). In somatic cells, this cycle includes four phases: G1, S, G2, and mitosis (M). During G1 phase, the cell grows as it prepares for DNA synthesis, S phase. Then in G2 phase, the cell grows in preparation for mitosis, in which the replicated DNA is equally divided into two newly formed daughter cells (Campbell et al., 2002).

Errors in the cell cycle are normally corrected during specific checkpoints at G1 to S, intra-S phase, and S to M. At these points, the cell cycle is temporarily arrested while regulatory enzymes ensure that there are no errors in the DNA sequence. If an error is found, the DNA damage is either repaired or the cell is tagged by a marker protein to commit suicide through apoptosis (Alberts et al., 2003). If inhibited, the cell cannot properly identify damage, and the cell cycle continues without the appropriate regulation (Kruh et al., 2000).

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The cell cycle can be perpetuated through two types of genetic mutations: oncogenes and tumor suppressor genes (Kruh et al., 2000). Tumor suppressor genes normally are involved with the repair of damaged DNA. Thus, whenever these genes are inactivated, damaged DNA is not properly repaired (Moossa et al., 1990). According to Ames and Gold (1991), every cell in the body experiences 10^5 DNA damaging events daily. Thus, the regulatory process of repairing DNA is an active and important process. Tumor suppressor genes can be broken down into two categories: caretakers and gatekeepers (Kruh et al., 2000). Gatekeepers have a direct roll in controlling cellular proliferation, while caretakers help preserve the integrity of the genome by preventing mutations from occurring. An inactivated caretaker does not lead directly to tumor initiation, but instead it causes genetic instability, which causes subsequent mutations. In contrast, inactivated gatekeepers play a more direct role in the tumorigenesis process (Kruh et al., 2000).

While tumor suppressor genes are dangerous when inactivated, oncogenes are only hazardous when active, at which point they are capable of inducing cancer in normal cells (McKinnel et al., 1998). Due to this, oncogenes are highly regulated in the body. Additionally, oncogenes have a wide variety of functions. For instance, some encode for growth factors that increase the proliferation of cells, others bind to DNA and regulate transcription, and yet others code for receptors or ligands involved in the cell cycle (Kruh et al., 2000). If over expressed, however, all of them can contribute to the development of cancer by promoting cell division (McKinnel et al., 1998).

Tumorigenesis, or tumor formation, is a multistep process requiring more than one active oncogene or inactive tumor suppressor gene. If a group of cells has a small number of these mutations, a benign tumor may form. These tumors lack the ability to metastasize or spread to other parts of the body. However, if the benign tumor has more mutations, it is possible for it to become malignant (McKinnel et al., 1998).

The process of carcinogenesis involves four steps. The first step is initiation, in which a carcinogen reacts with DNA causing a strand break or altering a nucleotide to form an adduct (McKinnel et al., 1998). Normally, a DNA polymerase repairs this problem, however, if the DNA replicates before the repair, the error can be permanently fixed into the genome (Kruh et al., 2000). Most errors of this type have no real effect on the body, but if a tumor suppressor is inactivated or an oncogene activated, the cell has a significant growth advantage, and the next step, promotion, may begin. During promotion, a molecule called a promoter causes selective proliferation, which may lead to the formation of multiple benign tumors (Alberts et al., 2003). Through one or more additional genetic alterations, the third step, known as progression, may occur. In this step, the tumor cells develop a significant growth advantage, which is so strong that they are able to break through the blood vessel membrane and travel to other areas through the process of metastasis. This actual conversion is the last step, and is referred to as malignant conversion (McKinnel et al., 1998).

This further establishes the importance of multiple mutated tumor suppressor genes and

oncogenes in cancer development. In other words, the growth advantage brought about by one mutation is not significant enough to overcome the natural immunity of the body. Tumors of this nature are contained because they are unable to break into the blood vessels (McKinnel et al., 1998). However, through multiple mutations, the growth advantage may be increased sufficiently to break through blood vessel membranes (McKinnel et al., 1998). For many years, scientists had no clue how to deal with this growth advantage. As a result, cancer was virtually untreatable, and even today, many types have no specific treatment.

Chemotherapy's potential to treat cancer was discovered during December of 1943, when an Allied warship holding mustard gas exploded (Williams, 2000). As a response to this, the army performed autopsies on the soldiers, which showed that their bone marrow had been destroyed by the gas, thereby inhibiting the production of red blood cells, white blood cells, and platelets. Accordingly, scientists hypothesized that the chemical may be used to fight cancer. To test this hypothesis, a chemical derived from mustard gas, known as mustine, was given to Hodgkin's disease patients and, even in some patients with late-stage Hodgkin's, the disease responded to the drug (Williams, 2000). In fact, this drug is still a key component of the MOPP (mustine, vincristine, procarbazine, and predinisone) regimen (Rüffer et al., 1998), which is one of the two primary treatments for Hodgkin's disease, the other being ABVD (Adriamycin, bleomycin, vinblastine, and dacarbazine) (Kennedy et al., 2003; Murphy et al., 1997).

Unfortunately, the treatments commonly used for cancer (radiation and chemotherapy) are both deleterious to the health of patients, and can actually cause death themselves by weakening the immune system and making patients more susceptible to other diseases (Schnell et al., 2003). The problem with these treatments is that they are not selective. That is, they act on all rapidly dividing cells causing the most recognizable symptom of cancer treatment: loss of hair. These treatments also inhibit the production of erythrocytes and white blood cells, causing patients to become anemic and neutropenic (Schnell, 2003). Anemia, a state of insufficient O_2 delivery to tissues, can cause problems with blood clotting, as well as lead to dizziness and lethargy. Neutropenia refers to a decrease in the number of neutrophils in the blood signifying a weakened immune system. When neutropenic, patients are more susceptible to secondary infections; even a common cold can be fatal. Furthermore, chemotherapy triggers neuroreceptors, such as those that bind dopamine and serotonin, which stimulate nausea and cause vomiting (Schnell, 2003).

Not only are chemotherapy and radiation dangerous, they also are not completely effective. According to Dr. Frank Balis, "We attribute our inability to cure many adults with more common forms of solid tumors to the ineffectiveness of chemotherapy to these diseases" (1998). In fact, the average five year survival rate among all cancers in the United States is only 63% (Jemal et al., 2005). Thus, newer and more effective treatments are being sought by scientists and pharmaceutical companies alike.

In the last few years, the field of nanotechnology has exploded as some scientists believe tiny objects known as nanoparticles may be able to help treat a variety of diseases, including cancer. By definition, nanoparticles can range in size from 1 to 100 nanometers (Cervellino et al., 2005). The nanoparticles being studied have a variety of compositions, shapes, and sizes. The most common composition includes either a carbon backbone or the presence of an inorganic metal, such as a gold (Zharov et al., 2003).

Recently, scientists have discovered that nanoparticles can easily enter cells. However, it is uncertain how this occurs. Dai et al. (2005) claims the influx of nanoparticles occurs by endocytosis. In contrast, Bianco et al. (2005) suggest the process happens through insertion and diffusion of particles through the lipid bilayer of the cell membrane. Furthermore and surprisingly, these particles can be linked to proteins, such as antibodies, and still enter cells (Dai et al., 2005). Fortunately, cancer cells express certain receptors that are not expressed by normal cells. Thus, nanoparticles attached to antibodies for these receptors can be directed to cancerous cells exclusively (Dai et al., 2005).

The ability of nanoparticles to selectively enter cancer cells has duel significance. Firstly, nanoparticles can work as drug deliverers. For instance, by linking certain proteins, such as tumor necrosis factor (TNF), a protein with known antitumor activity, to the particles a new mechanism for fighting cancer can be utilized (Paciotti et al., 2004). Secondly, nanoparticles have been shown to absorb different wavelengths of light than the body, and when exposed to appropriate wavelengths nanoparticles heat up, but the body does not. This method, known as hyperthermia, can be used to selectively kill cancer cells by heating nanoparticles that are linked to antibodies (Ito et al., 2003a).

The specificity of these techniques is key, because unlike the deleterious effects of chemotherapy and radiation, treatment with nanoparticles should result in no major side effects. Furthermore, in preliminary studies, hyperthermia and drug delivery have both been successful, and currently, both hyperthermia and drug delivery are being heavily investigated as treatments for cancer (Dai et al., 2005; Onishi et al., 2003). The purpose of this review is to discuss the nanoparticle techniques of hyperthermia and drug delivery and determine whether they may one day replace the current techniques of chemotherapy and radiation as a treatment for cancer.

Imaging to Detect Cancer Cells

Beyond having the power to treat cancer, nanoparticles may also be used to detect the disease. Moreover, some therapies hope to utilize hyperthermia in such a way that diagnosis and treatment can occur together. There are several techniques scientists are investigating to improve cancer detection and couple it with hyperthermia (Loo et al., 2004).

One popular technique involves attaching bioconjugates, such as antibodies, to the nanoparticles. Loo et al. (2005) attempted to analyze this technique by utilizing the tendency of breast carcinoma cells to overexpress the HER2 biomarker. Thus, by conjugating an antibody of HER2 to a PEG linker complex, which enhances biocompatibility and blood flow, and then attaching the complex to a gold nanoshell, the particle is linked exclusively to breast cancer cells (Loo et al., 2005).

Using this, Loo et al. (2005) cultured three types of cells: cells with the anti-HER2/PEG/nanoshell complex, cells with a non-cancer specific antibody, and cells without nanoshells. These cells were viewed with

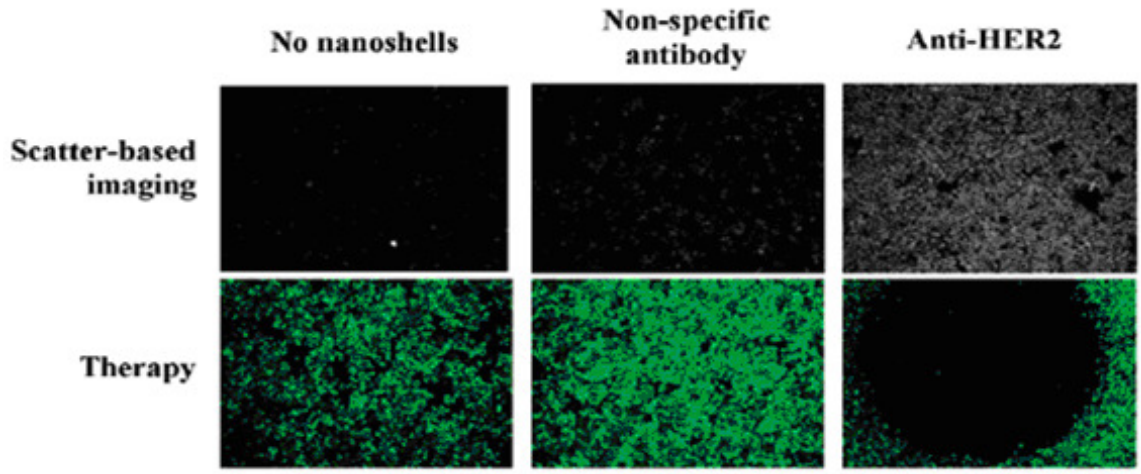

Figure 1. Imaging and hyperthermia using nanoparticles. Imaging and therapy of SKbr3 breast cancer cells using HER2 linked nanoshells. Top row: darkfield imaging of of HER2 expression based on light scattering. Bottom row: cell viability assessed through calcein staining with exposure to ~820 nm near infrared (NIR). Cell death was observed only in cells treated with anti-HER2 nanoshell take from (Loo et al., 2005).

a darkfield microscope sensitive to scattered light, and only the Anti-HER2 cells showed much light scattering (Figure 1). In contrast, the cells with the non-specific antibody showed some light scattering, but it was not as dense. This illustrates that the Anti-HER2 treated cells attached exclusively to cancer cells, and exposure of light identified cancer cells. Furthermore, when treated with near-infrared (NIR) light of around 800 nm, cytotoxicity was observed only in the presence of the cells treated with Anti-HER2 nanoshells (Figure 1) (Loo et al., 2005). Thus, the hyperthermia treatment was successful, but only with the Anti-HER2 treated cells.

Hyperthermia to Kill Cancer Cells

As mentioned earlier, hyperthermia is the killing of cells through the heating of nanoparticles. One of the problems of hyperthermia is containing the heat in such a way that it does not affect other cells. To combat this, scientists use specific types of nanoparticles for hyperthermia, such as magnetite cationic liposomes (MCLs) (Kobayashi et al., 2005). These spherical particles contain a positively charged phospholipid exterior that interacts with the negatively charged cell surface, easily entering cells. The inside of the MCLs is a 10 nm magnetite nanoparticle (Kobayashi et al., 2005). Additionally, these particles have maintained the ability to bind to antibodies and can provide tumor-specific contrast enhancement.

Gene Therapy/Hyperthermia Combination

Hyperthermia appears to be effective in some cases by itself, however, in advanced stages of several types of cancer, such as melanoma, it may not be sufficient (Ito et al., 2003a). Furthermore, to treat cancer, hyperthermia requires many treatments. However, in conjunction with other processes, scientists hope to find a way to use one round of hyperthermia to eradicate the disease. The combination therapies revolved around the use of substances to boost anti-tumor immunity. Thus, in addition to hyperthermia, the cancer cells will be assaulted by a revamped immune system (Ito et al., 2003b). Ito et al. (2003a) have been analyzing the use of one such protein, heat shock protein 70 (HSP70), in conjunction with hyperthermia with MCLs. Expression of this protein protects cells from heat-induced apoptosis (Mosser et al., 2000), but recently, it has also been shown to be a key component in immune reactions (Srivastava et al., 1998).

To analyze HSP70 gene therapy combined with hyperthermia, Ito et al (2003a) analyzed how mice with malignant melanoma reacted to tumors that had been given a plasmid containing human-inducible hsp70 complimentary DNA. The primary finding was that hsp70 gene transfer successfully boosted the immune system during hyperthermia (Ito et al., 2003a). They determined this by comparing tumor size after exposure to hsp70 containing plasmid, hyperthermia, and the combined treatment. Both treatments alone showed improvement, but in each case, additional treatments would be required because the tumors began to grow again at around the tenth day. The combined therapy, however, completely eradicated cancer in 3 of the 10 mice with only one treatment. Because hyperthermia can be used multiple times without any negative effects, it is believed that the cancer could have been eradicated in the other mice with subsequent treatments. Moreover, tumors with the combined therapy were 16 times smaller than the hyperthermia only treated tumors after thirty days, and 24 times smaller than the tumors given hsp70 (Ito et al., 2003a).

Hyperthermia with Dendritic Cell Addition

The use of immune triggering proteins is not the only way to boost anti-tumor activity. For instance, mature dendritic cells (DC) are an integral part of a normal immune response, which stimulate the growth of $CD4^+$ T cells, $CD8^+$ cytotoxic T lympocytes, and natural killer cells (Palucka et al., 1999). Unfortunately, mature DCs cannot take up antigen, and thus addition of these cells would not result in the proper immune response. Injection of immature DCs, however, has been reported to cause antitumor activity (Celluzzi et al., 1998).

Tanaka et al. (2005) decided to go straight to the source by actually adding additional dendritic cells (DC) after mouse EL4 T- lymphoma tumors were treated with hyperthermia. While only 1 in 8 of the mice

treated with hyperthermia alone had complete tumor regression, 6 in 8 of the mice treated with hyperthermia and immature DCs had complete tumor regression. Based on this, it appears the tumor cells killed by hyperthermia release antigen proteins which the immature DCs take up and are then presented to T cells via MHC class I and/or II antigens (Tanaka et al., 2005).

Drug Delivery Using Nanoparticles

Drug delivery is the carrying of drugs using nanoparticles specifically to the cells causing the disorder. In the case of cancer, these drugs are frequently known chemotherapeutic agents. Intravenously, these drugs cause a variety of side effects. However, by linking them to nanoparticles the drugs go directly to the source and do not affect healthy cells (Paciotti et al., 2004). As is the case with hyperthermia, certain types of nanoparticles are better adapted for drug delivery than others. For instance, nanoparticles composed of colloidal gold easily attach various drugs. Colloidal gold is a dispersed solution of nanoparticles of Au^0 (Paciotti et al., 2004). Additionally, polybutyl cyanoacrylate (PCB) nanoparticles attach drugs, protect them against enzymatic degradation, reduce their toxic effects, and limit distribution of the drug outside the target area (Reddy et al., 2004a).

Tumor Necrosis Factor and Colloidal Gold

Tumor necrosis factor (TNF) is a cytokine that affects coagulation, lipid metabolism, insulin resistance, and proper function of endothelial cells (Paciotti et al., 2004). It is produced during immune response primarily by monocytes and macrophages and has the ability to induce death in tumor cells (Elliott et al., 1994). Unfortunately, TNF causes systemic toxicities that have prevented it from being used as an anti-cancer drug (Furman et al., 1993). This toxicity can be attributed to rapid uptake of TNF by the reticuloendothelial system (RES) (Paciotti et al., 2004). Through the use of colloidal gold nanoparticles, Paciotti et al. (2004) were able to construct a vector which can avoid detection and clearance by the RES. Thus, the nanoparticles (PT-cAu) delivered TNF specifically to tumor cells, eliminating the associated systemic toxicity.

Next, Paciotti et al. (2004) compared treatment using native TNF and PT-cAu-TNF which showed both reduced tumor size in a concentration dependent manner. However, mice given 12 μg native TNF suffered 25% fatality and all given 24 μg native TNF died whereas none of the mice treated with PT-cAu-TNF perished. Furthermore, Figure 2b illustrates that while 15μg of Native TNF has approximately the same affect on tumor size as PT-cAu-TNF through 16 days, the survival rate using the native form is 40% lower. Thus, without the colloidal gold nanoparticles, TNF is extremely toxic. These nanoparticles help TNF circumvent the RES and enter selectively into cancer cells, which ultimately causes tumor cells to die (Paciotti et al., 2004).

Localized Chemotherapy

As mentioned earlier, the main problem with chemotherapy is that it is not tumor specific. Thus, chemotherapy drugs tend to act on all rapidly dividing cells. Through the use of nanoparticles, however, the same drugs can be linked specifically to cancer cells at higher concentrations for longer periods of time. Thus, the drugs not only have increased cytotoxic activity, but also adverse side effects are limited (Alberts et al., 1985).

Doxorubicin

Doxorubicin hydrochloride (Dox), also known as adriamycin, is a cytotoxic anthracycline that is an essential component of chemotherapeutic regimens used to treat acute lymphoblastic leukemia, breast carcinoma, Hodgkin's and Non-Hodgkin's lymphoma (Murphy et al., 1997). The drug works by halting DNA replication, and thereby preventing further proliferation of the disease (Reddy et al., 2004a).

Fortunately, Dox's anti-tumor activity has been widely documented, and there is no reason to think it would behave differently if attached to a nanoparticle. At the same time, intravenous treatment of Dox causes systemic toxicity that can cause severe diarrhea, neutropenia, anemia, hair loss, and heart damage. Thus, scientists are investigating the use of different types of nanoparticles that can be used to deliver Dox directly to cancer cells, ultimately preventing systemic toxicity (Wilkes et al., 2000).

Reddy and Murthy (2004a) investigated this by analyzing two different polymerization techniques for making polybutyl cyanoacrylate (PRC) nanoparticles: dispersion polymerization (DP) and emulsion polymerization (EP). The result of each polymerization technique produced structurally similar molecules. The difference, however, was that the EP nanoparticles were smaller. Therefore, Reddy and Murthy (2004a) sought to find out whether the size difference of the PRCs affected the nanoparticles' ability to deliver Dox. They found that EP particles provided a longer half-life of Dox in the blood and a lower tissue distribution, which is consistent with their previous finding that EP nanoparticles have enhanced permeability and retention effects (Murthy and Harivardhan, 2003). Conversely, DP nanoparticles were quickly cleared into the RES. Both techniques demonstrated a significant increase in bioavailability of Dox compared to intravenous injection of Dox solution (Reddy and Murthy., 2004a). Together, the experiment identified the EP nanoparticles as a potential method of improving Dox therapy by reducing systemic toxicity (Reddy and Murthy, 2004a).

Following the polymerization study, Reddy et al. (2004b) examined the affect of Doxorubican loaded poly(butyl cyanoacrylate) (DPBC) nanoparticles on Dalton's lymphoma. They found that the DPBC nanoparticles sequestered in the tumor after subcutaneous injection much better than did free Dox. Additionally, they noted that there was a low amount of Dox found in the heart from the DPBC nanoparticles, and confirmed that Dox delivered by DPBC nanoparticles has an increased retention time within tumors. This confirms the results of the previous experiment, and also shows that cardiac toxicity may be limited through this technique.

Ma et al. (2004) developed another type of nanoparticle to be used for Dox delivery to tumor cells. The particles, known as carbon magnetic nanoparticles (CMNP), were created using a new technology known as dense medium plasma (DMP) technology. The particles consist of a carbon-based host structure with iron and iron oxide particles evenly dispersed (Ma et al., 2004). The CMNP-Dox and intravenous free Dox were applied to osteosarcoma cells to test antiproliferative activity. The results showed that at the highest dose,

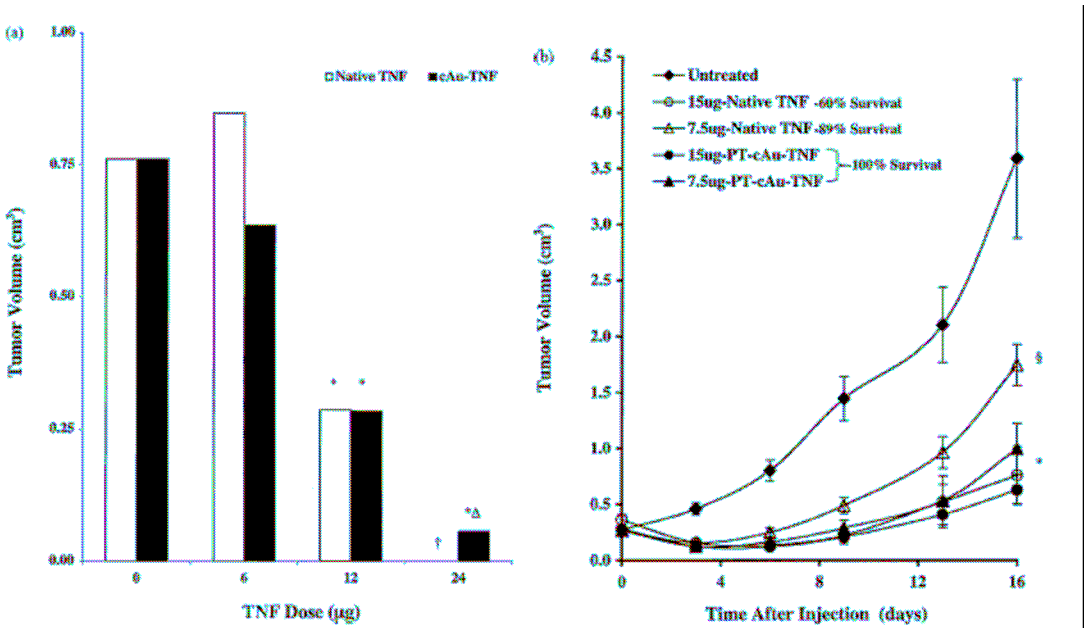

Figure 2: TNF effect on tumor volume in mice MC-38 colon carcinoma tumors.
a.) Antitumor efficacy of native TNF and the cAu-TNF vector. Mice with MC-38 colon carcinoma tumors were intravenously injected with increasing concentrations of native TNF of cAu-TNF vector (n=4/group/dose). Tumors were measured 10 days after treatment using three dimensional measurements (L x W x H). b.) Antitumor efficacy of native TNF and PT-cAu-TNF vector using one group as a control. Two groups with either 7.5 or 15 □g of intravenously injected PT-cAu-TNF. Another two groups were intravenously injected with 7.5 or 15 µg of native TNF. The size of tumors were then measured on various days (Paciotti et al., 2004).

free Dox had no significant effect on the tumor cells compared to CMNP-Dox, which completely stopped proliferation at 120 µg/ml Dox. Interestingly, at 240 µg/ml, CMNP-Dox had a reduced effect, believed to be because of steric hindrance caused by excess nanoparticles (Ma et al., 2004). One of the chief advantages of this system, however, is that it can be made in one step under atmospheric pressure using inexpensive chemicals, such as benzene and acetonitrile, making it both effective and cost efficient (Ma et al., 2004).

Paclitaxel

Paclitaxel is a chemotherapy drug that can be used to treat Kaposi's sarcoma and metastatic breast, ovarian, and bladder cancer (Wilkes et al., 2000). It is an anti-microtubule compound that prevents continuation of the cell cycle and thus proliferation (Wientjes et al., 2004). In the case of bladder cancer, doxorubicin and mitomycin C are ineffective treatment options due to their inability to pass through the transitional epithelium in the wall of the bladder known as the urothelium. Since paclitaxel is lipophilic, however, it can freely pass through the urothelium (Wientjes et al., 2004). The FDA approved formulation for paclitaxel includes the solvent Cremophor. Cremaphor causes paclitaxel to become entrapped in the micelles of the bladder, which lowers the drugs ability to penetrate the urothelium (Knemeyer et al., 1999). To combat this, Wientjes (2003) used DMSO as a surface-active agent that disrupted Cremaphore micelles and enabled paclitaxel to be delivered to the tumors; however, this technique caused increased urine production and associated drug removal. Consequently, with less time in contact with the cancerous cells, paclitaxel was less effective.

Wientjes's et al. (2004) second attempt to facilitate the transfer of paclitaxel through the urothelium utilized gelatin nanoparticles loaded with the drug. These nanoparticles are hydrophilic and thus uptake fluid rapidly allowing for paclitaxel to be released easily. This is important because the quicker the drug is released, the longer its exposure to cancer cells before urination. The concentration of paclitaxel in the urine, which was collected during treatment, was 2.6x that of the cremophor/EtOH formula. Additionally, 87% of the drug was released in two hours (Wientjes, et al., 2004), compared to only 45% after 3 days for paclitaxel-loaded poly(ethylene oxide)- poly (lactide/glycolide) nanospheres used to regulate smooth muscle cell regulation (Suh et al., 1998). In summary, paclitaxel loaded gelatin nanoparticles were able to penetrate the urothelium of the bladder and rapidly release the drug, making them a promising treatment for bladder cancer (Wientjes et al., 2004).

Gene Delivery using Nanoparticles

Nanoparticles can deliver proteins with anti-tumor activity into tumor cells and additionally, they can be used to deliver chemotherapeutic drugs directly to tumors, avoiding systematic toxicity. The versatility of these small particles also allows them to transport plasmid DNA with tumor suppressor genes to tumor cells. This causes a tumor suppressing protein to be produced which induces tumor cell apoptosis, effectively fighting the cancer (Ramesh et al., 2004).

MDA-7

First identified in human melanoma cells (Jiang et al., 1995), the human melanoma differentiation associated gene 7 (*mda-7* or *IL-24*) is a tumor suppressor gene. In late stage human melanoma, MDA-7 protein is absent, whereas in early stage melanoma it is present. Accordingly, this gene

product is likely involved with progression of the disease (Ellerhorst et al., 2002). Furthermore, the protein is absent in a variety of human tumors including lung, breast, and colorectal carcinomas and sarcomas, and thus, it is believed to be involved in both the development and progression of these human cancers (Chada, et al., 2003).

Previous studies have shown that through using adenoviral vectors, expression of MDA-7/IL-24 triggers cytotoxic related cell death and growth suppression in several human cancer cells (Ramesh et al., 2004). Moreover, normal cells are not affected by exposure to *mda-7*gene, making it a potentially strong anti-tumor therapy. In 2003, Chada et al. used an adenoviral receptor to deliver *mda-7* to tumors in the lungs. The results were promising, because this procedure caused expression of MDA-7 induced apoptosis in the tumors. Unfortunately, the adenovirus vector can cause an immune response and liver toxicity (Vlachaki et al., 2002). Therefore, a new vector for *mda-7* delivery to disseminated cancers is needed.

Ito et al. (2003c) demonstrated that DOTAP: cholesterol nanoparticles can transport tumor suppressor genes to tumors in the lungs and increase the transgrene expression of these genes. Based on this, Ramesh et al. (2004) tested the use of cationic DOTAP: cholesterol (Chol) nanoparticles as a vector for delivery of *mda-7* gene. They found that cells treated with the DOTAP/mda-7 gene showed significantly fewer tumors (Figure 3). Additionally, they found no resistance to multiple treatments with this therapy, as well as no systematic toxicity. Furthermore, the treatment was still successful in immunodeficient and immunocompetent organisms. Thus, using DOTAP: Chol nanoparticles as a vector for the *mda-7* gene is a novel approach for cancer therapy that shows much promise (Ramesh et al., 2004).

Discussion

In this paper, I have chronicled three promising techniques for treatment of cancer using nanoparticles: hyperthermia, drug delivery, and gene therapy. These techniques each have several advantages over the current treatments of radiation and chemotherapy. Firstly, neither of these treatments causes systematic toxicity. In fact, both hyperthermia and drug delivery can be directed specifically to cancer cells. Ultimately, this is advantageous because it greatly reduces the physically and psychologically demanding side effects of chemotherapy and radiation, which include, but are not limited to anemia, neutropenia, hair loss, diarrhea, sterility, and nausea.

These side effects are thought to be worthwhile because of chemotherapy's effect on cancer, but all cancer cells are not responsive to chemotherapy. Furthermore, some cancers develop resistance to chemotherapeutic drugs (Gottesman, 2002). There are several reasons for this. As mentioned in the beginning, tumor cells have a variety of mutations, and all tumor cells do not have the same mutation. Some mutations allow cells to randomly develop resistance to drugs because they no longer express the protein receptors to which the drug interacts. Thus, the cells without the receptor have a growth advantage, and if another drug is not used, these cells will proliferate rapidly (Gottesman, 2002). Additionally, tumor cells may produce more target proteins than the drugs can bind. Since chemotherapeutic agents are not specific, the concentration of the drugs cannot be raised, as other systems of the body would be effected as well (Gottesman, 2004). Furthermore, enhanced amplification of the *MDR1* (Multiple Drug Resistance) gene results in the encoding of a large transmembrane protein which can stop certain drugs from entering a cell and also eject drugs already in it (Bredel et al., 2002).

With chemotherapy, any form of resistance requires another type of drug; however, nanoparticles may hold the key to circumventing such resistance. Early trials with hyperthermia and gene delivery show that each technique may be used multiple times. Hyperthemia, for instance, does not work on hindering processes inside the cell, but instead, it heats the cell up to such high temperatures that it denatures proteins and DNA (Dai et al., 2005). Heat shock proteins that stabilize proteins to prevent denaturing are themselves denatured when exposed to heat of this magnitude (Ito et al., 2003). Thus, hyperthermia can be done

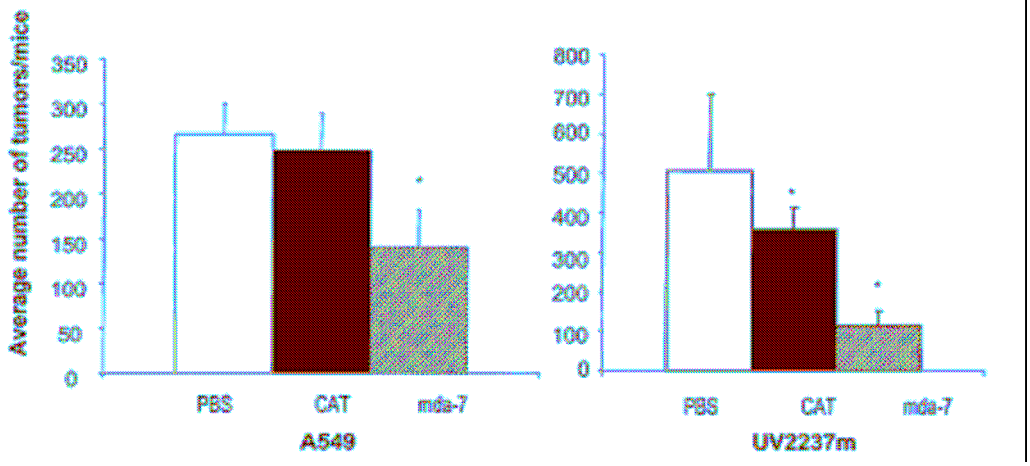

Figure 3- Mice treated with *mda-7* exhibit a lower number of tumors.
Mice with A549 and UV2237m lung tumors were treated daily for a total of six doses (50 □g/dose) with phosphate-buffered saline (PBS), DOTAP:Chol-chloramphenicol acetyl transferace (CAT) nanoparticles, or DOTAP: Chol-*mda-7* nanoparticles. Tumor growth was only inhibited by DOTAP:Chol-*mda-7* nanoparticles ($P<0.05$; Ramesh et al., 2004).

repeatedly without detrimental effects to other systems or the threat of tumor cells becoming resistant to it (Dai et al., 2005). Moreover, through gene delivery, expression of tumor suppressor genes inside tumors can be controlled, so in essence, tumors are forced to fight themselves. Early experiments suggest, that using the mda-7 gene in this manner can be performed repeatedly and cells develop no resistance (Ramesh et al., 2004).

While hypothermia and gene therapy appear to circumvent resistance, through the use of higher drug concentrations and exposure time, localized drug delivery provides another option. The use of nanoparticles allows for higher concentrations of drugs, such as native tumor necrosis factor and doxorubicin, to be used. This is possible because the nanoparticles specifically target cancer cells, and thus, there will be no associated systemic toxicity. Because a higher amount of the drugs can be used, the initial treatment has a larger effect, as more of the drug is able to interact with the tumor cells. Additionally, a second treatment can be administered much more rapidly afterward, since the rest of the body does not have to recover. Together, there is a much smaller chance that the tumor would develop resistance, because treatment can take a shorter period of time. However, it is possible that some of the tumor cells have innate resistance, in which case, another drug would have to be used.

In summary, the techniques of drug delivery and hyperthermia using nanoparticles have the potential to decrease side effects while increasing the cure rate of cancer patients. These techniques promise a substantial improvement over chemotherapy and radiation. Over the next few years, if the research conducted on nanoparticles continues to find promising results, the treatment of cancer all over the world may be substantially altered. The cure for cancer may in fact be close at hand.

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