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MEETING REPORT

Nanotechnology in Early Detection of Cancer

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Nanotechnology is defined as the creation of functional materials, devices, and systems through control of matter at the scale of 1 to 100 nanometers and exploitation of novel properties and phenomena at the same scale. Advances in nanotechnology are thought to be the impetus for the "Next Industrial Revolution" by the National Nanotechnology Initiative. One potential application highlighted in the above Initiative is the detection of emerging diseases as a means for shifting focus from patient care towards early detection and prevention.

Nanotechnology offers a novel set of tools for early detection of cancer. At a recent workshop organized by the National Cancer Institute (NCI) and the National Institute of Standards and Technology (NIST) on "Nanotechnology in Early Detection of Cancer," nanotechnology experts from academia, industry, and government institutions discussed the state of the art and explored the potential utility of nanotechnology for early cancer detection. Scientists active in the field also explored the impact of nanotool applications in cancer research.

The workshop was held at the National Institute of Standards and Technology campuses on August 30 and 31, 2001 and was chaired by Drs. Lee Hood and George Whitesides. Topics included the use of lasers to measure optical deformability in cancer cells, detection, sensing and therapeutics through the use of nanopores and nanomaterials, molecular combing to detect genomic instability, molecular nanomechanics for detection of biomolecular interactions, dendrimers, nanodevices and nanotechnology platforms for sensing, delivery, and therapeutic applications. It was evident from the workshop that nanotechnology has the potential to make significant contributions to prevention and detection in addition to diagnosis and treatment of cancer. The consensus was that nanotechnology offers important new tools for detection where existing and more conventional technologies may be reaching their limits. Nanotechnology was identified as a possible means to provide direct readout of genomic and proteomic information both at the single cell and single molecule level. Its utility in analyzing and characterizing extremely limiting biological samples was also discussed. This commentary summarizes the highlights of the workshop and the recommendations made by the leading proponents of nanotechnology.

Nano defines the structure of biomolecules whose dimensions are intrinsically in the nanoscale and is the venue where changes are generally initiated during the earliest phases of the disease process. One advantage of nanotechnology is that nano-sized tools are small relative to the size of a cell and hold great promise for developing effective techniques to work within cells or even at a subcellular level. The present obstacle to early detection of cancer lies in the inability of existing tools to detect these molecular level changes directly during early phases in the genesis of a cancer. Nanotechnology is a potential tool that could help detect the molecular changes and assist in focusing on preventive efforts.

Workshop Objectives

The goal of the workshop was to bring together specialists from government, academia, and industry to discuss state of the art and explore the potential utility and applications of nanotechnology for early detection and prevention of cancer. The workshop integrated invited presentations from scientists actively interfacing nanotechnology with biology and clinical applications. An open forum for active discussion was encouraged through panel discussion and two concurrent breakout sessions on nanotechnology devices for early detection of cancer and nanotechnology platforms for high-throughput identification of biomarkers.

The workshop opened with a keynote lecture by Dr. George Whitesides (Harvard University) wherein he pointed out that nano-sized tools are small relative to the size of cells and hold promise for developing effective techniques of working within cells or for

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manipulations at a subcellular level. At the nano scale, a wide range of structures, derived from biology, are obvious candidates for inclusion in a tool kit for developing nanoscience applications useful in cancer. He referred to nanoelectronics and information technology as the driving forces of nanotechnology, which be approached either in a top-down or a bottom-up manner. While discussing potential nano-applications, Dr. Whitesides talked about lesion detection using magnetic resonance imaging with magnetic iron colloid nanoparticles, wrapped in a carbohydrate matrix to encourage uptake by cells. He also described quantum dot-based detection systems that could act as intracellular markers in marking specific portions of cells. In his concluding remarks, Dr. Whitesides stated that arrays, single molecule measurement, metal colloids, imaging agents, cell isolation methods, and drug development technology are some of the areas in cancer detection and therapy that might exploit recent nanotechnological advances.

Interface Between Biology and Nanotechnology

Optical Deformability Dr. Kas (University of Texas, Austin), described the "optical stretcher" that his group has developed to diagnostically deform cells. Cellular deformability, a function of cellular elasticity, is an inherent property of the cytoskeleton and is often used as a marker to identify cancerous cells and indicate different stages of neoplasia. Mechanical properties of the cytoskeleton are important in cellular motility and are good indicators of phenotypic changes such as those that occur during different phases of neoplasia.

Dr. Kas explained that each cell has a cytoskeleton, an active, highly dynamic packaging material that gives the cell mechanical stability as well as shape. He explained that the three main types of polymers found in the cellular cytoskeleton are microtubules, actin filaments, and intermediate filaments. The cytoskeleton plays a role in cell motility, development of RNA and ribosomes, and protein expression. Between 5% and 10% of the energy in cells is dedicated to the turnover of the cytoskeleton, a phenomenon known as "treadmilling." Cell elasticity responds nonlinearly to changes in the concentration of actin filaments. Furthermore, he explained, changes in cytoskeleton are functional to different phenotypes of cancer.

The optical stretcher incorporates two optical fibers into a microfluidic flow chamber that can induce cells to stretch by generating a force of 800 piconewtons. As the laser light hits cells, virtually all passes through without absorption or reflection, causing cells to stretch. He demonstrated that red blood cells, leukemia cells, and normal human neutrophils could be distinguished by this technique. In collaboration with The University of Texas M.D. Anderson Cancer Center, Dr. Kas and colleagues are exploring the potential of the optical stretcher to detect cancerous and precancerous exfoliated cells from the cervix obtained by thin preps and to correlate these results with cancer progression. One goal of this research is to determine whether the technique can distinguish between different types of dysplasia and whether it can be correlated with human papilloma virus infection for the early detection of cervical cancer.

Nanopores for Detection of Biomolecules Dr. Kasianowicz (NIST) discussed development of a single nanopore for ultrarapid DNA sequencing. As DNA passes through the nanopore, which is one base in diameter, the change in conductance across the channel is used to identify which base is passing through the pore. Scientists have been able to examine the changes in conductance of a nanopore and detect analytes in a solution. He suggested that new, nanoscale technologies for cancer detection could be developed using nanopores. He explained that polymer transport through nanopores occurs naturally in a variety of organisms and cells.

Dr. Kasianowicz and colleagues are studying how different types of polymers are transported through single-protein ionic channels formed by the α-hemolysin (αHL) molecule, which is secreted by *Staphylococcus aureus*. He discussed how the characteristics of current, such as fluctuations, variance of fluctuations, and spectral densities, could be used to determine which molecules bind or pass through the nanopore. His laboratory is also developing single nanopore-based sensors for simultaneous multianalyte detection.

Molecular Combing for Detecting Genomic Instability Dr. Bensimon and colleagues (Pasteur Institute) have developed a technological platform using a method known as molecular combing, which straightens and aligns DNA molecules on a solid surface, for the study of genetic alterations. He explained that this method could be used to study the organization of rearranged regions in the genome and determine how this region evolved through the carcinogenesis process. The investigators also used the single DNA molecule approach to quantitatively study the dynamics of DNA replication. Molecular combing relies on the action of a receding air/water interface or meniscus. The force applied by the meniscus on the DNA molecule is localized in the immediate vicinity of the air/water interface. The resulting stretching is uniform and reproducible, and the extension of polymeric molecules like DNA is linear, constant, and proportional to their number of base pairs. Large amounts of DNA (more than 700 kb) can be stretched, and molecules once combed this way can be denatured, hybridized, and visualized using fluorescent hybridization probes.

Underlying mechanisms in carcinogenesis such as microdeletions, inversions, and amplification of genetic loci can be detected using this approach. Molecular combing enables the visualization and microdissection of a genomic region, which can provide a molecular genomic classification of a tumor as well as its evolution over time.

Molecular Nanomechanics Dr. Majumdar (University of California, Berkeley) talked about the use of microcantilever beams for detecting molecular interactions. He explained that biomolecules, by virtue of their

flexibility, effect surface stress changes upon binding, which can be measured. He reported that microcantilever beams can be used to detect biomolecular interactions through measurement of stress changes. When specific biomolecular binding occurs on the surface of a microcantilever, intermolecular mechanics cause changes in surface tension that create torque at the nano level and bend the cantilever. This bending phenomenon can be measured optically.

Dr. Majumdar noted that this phenomenon has been used to detect biomolecules such as DNA and proteins. He described experiments in which cantilever beams, 200 to 300 microns in size, are placed in fluid cells that are injected with molecules and liquid solution. The bending motion of the cantilever beams is detected down to the nanometer level using a laser and a position-sensitive dye. This technique was sensitive and specific enough to quantitatively detect single base pair mismatches in DNA and cancer-induced antigen-antibody binding. He described how this technology could be applied to detecting prostate-specific antigen levels.

Once the surface stress is calculated, the prostate-specific antigen concentration in solution can be determined based on the surface stress. His group has used this protocol to successfully detect two forms of prostate-specific antigen over concentrations ranging from 0.2 to 60 mg/ml in the background of human serum albumin. Similarly, his group has demonstrated that this technology could be used as a platform for label-free biomolecular detection of DNA hybridization, protein-protein interactions, DNA-protein interactions, and DNA-ligand binding. He mentioned that the challenge would be to develop an array to measure hundreds of cantilevers simultaneously with a resolution of 1 nm.

Integration of Engineered Biosystems Dr. Neves (Cornell University) described his group's research efforts to incorporate biological complexity into fabricated devices. He demonstrated that the F_0F_1 ATPase is a natural candidate for acting as a biomolecular motor because it can synthesize or hydrolyze ATP. He discussed the group's efforts in generating a hybrid nanosystem by harvesting the F_1 portion of the ATPase and attaching a nickel propeller to it. The result is a hybrid nanosystem consisting of the F_1 ATPase biomolecule assembled onto a nanofabricated substrate with a nickel propeller attached to its gamma subunit. Such a device could be controlled by chemical switches and targeted to attack pathogens such as viruses, collect them, and withdraw from the cell. The challenges facing this group include producing and engineering stable proteins in large quantities, fabricating structures that are physically compatible with these proteins and are chemically active.

Interface Between Clinical and Nanotechnology Applications

Biomedical and Therapeutic Applications Dr. Baker (University of Michigan) described the work of his group on the development of an integrative device that could target the tumor, sense for pathophysiologic defects either in RNA, DNA, or protein, select a therapeutic based on these characteristics, induce noninvasive internal treatment to release the therapy, document the response, and identify residual tumor cells. Their work centers on the dendritic polymer, characterized as a molecule that is a synthetic polymer, which is uniform, reproducible, and close in size to proteins.

Dr. Baker and colleagues have developed dendritic polymeric nanodevices that can detect cancer cells, identify cancer signatures, and provide targeted delivery of anticancer therapeutics (ie, cis-platin, methotrexate, and Taxol) and contrast agents to tumor cells. He and his colleagues have demonstrated that after 3 minutes, these polymers showed uniform uptake in cells, and the uptake is faster than that seen with most antibody-mediated deliveries. The dendrimers survive for more than 2 weeks inside the cells and do seem to be affected by the body's multidrug resistance processes. When given intravenously to mice, specific receptor-mediated binding and internalization within tumor cells were observed. The size of these polymers is under the filtration threshold of the kidney, and they cannot cross the blood-brain barrier. Since there is nothing for the host's T cells to bind to, there is no immune reactivity.

Nanotubes and Nanowires for Molecular Detection Dr. Lieber (Harvard University) talked about carbon nanotubes and nanowires for developing imaging tools and sensors, respectively. The chemical morphology and electronic properties of these well-defined inorganic materials can be manipulated to help detect and sense single molecules. Carbon nanotubes have been helpful in improving the resolution of atomic force microscopy measurements at the molecular scale. He discussed the potential for using this technology to scan down DNA and look for single nucleotide polymorphisms and described haplotyping experiments using peptide nucleic acid probes. In principle, it should be possible to detect whether an individual has a high-risk or low-risk configuration for developing the processes that lead to cancer. This technique can serve as an alternative to PCR and identify multiple nucleotide polymorphic sites in large strands on nonamplified DNA at relatively high throughput and low cost.

Dr. Lieber noted that semiconductor nanowires have the same dimensions as DNA or carbon nanotubes. Unlike carbon nanotubes, nanowires have a great deal of chemical flexibility, which allows for the development of designed electronic properties. Nanowires could be switched on and off using gate voltage and act as field-effect transistors. The binding of chemical or biological species to the surface of a nanowire results in depletion or accumulation of carriers. The charge in carrier concentration, due to binding, can be directly monitored through measurement of the nanowire conductance. This property allows for real time detection and monitoring of ligand-receptor binding and protein binding.

Nanodevices for Intracellular Sensing Dr. Philbert (University of Michigan) discussed real-time reversible chemical sensing and strategies for the early detection of cancer and for light-based therapy using PEBBLE (Probes Encapsulated by Biologically Localized Embedding) nanosensors. These spherical devices consist of sensor molecules entrapped in a chemically inert matrix; they have the ability to carry out real-time sensing of ions and small molecules. This technology can be readily combined with routine confocal microscopy. He outlined the application of this technology for quantitative intracellular oxygen sensing using ratiometric PEBBLEs. These nanoparticles can perform ratiometric measurements using two or more components. Inserting glucose oxidase in PEBBLEs to consume oxygen allows for measurement of the oxygen tension within the PEBBLE as well as the measurement of the glucose concentration. PEBBLEs have been used to measure analytes such as calcium, potassium, sodium, nitric oxide, and chloride.

Dr. Philbert's group also focuses on using PEBBLE technology for the early detection, diagnosis, and treatment of cancers such as intercranial tumors, or gliomas, which have a built-in exclusion mechanism afforded by the blood-brain barrier. Nanoparticles can cross this barrier, but the challenge is to develop a nontoxic yet therapeutically viable device, protected from immune system functions, proteases, and other biochemical events that might destroy it. He explained that the intent is to deliver the particle in close proximity to the tumor and empty the particles into the tumor to cause targeted cellular damage. Prototype studies are in progress involving injections of 20-nm particles into the tails of rats that had tumors similar to high-grade brain tumors in humans.

Single Platform Nanodevices Dr. Mauro Ferrari (Ohio State University) discussed a single-platform for integration of diagnostics and therapeutics on the same technological foundation as well as "smart" therapeutic interventions that are site-directed, immunoguided, self-regulated, or remotely controlled. Work in his group involves the development of micro- and nanotherapeutics for implantable nanopumps, smart contrast agents, and trans-tissue patches. Developing smart contrast agents was perceived as an immediate opportunity for nanotechnology to have a clinical impact.

Dr. Ferrari described the evolution of a nanopore membrane therapeutic device that allows for adjusting the thickness, height, and width of the nanopore, which could be used for immunoisolating cells, cell factories, and implantable sensors. His group has developed nanopore biocapsules for use as implantable hybrid drug factories that hold promise in early detection of cancer through their use as biomolecular signal amplifiers. One key function is their ability to act as a drug dispenser with controlled long-term release of biological molecules. He explained how prototype implantable nanopumps deliver therapeutics and noted that they could be used in patients at high risk of first onset or recurrence of disease.

Dr. Ferrari's group also has developed particles less than 1 micron across that have functions in targeting endothelial cells and particle immunoconjugation to interfere with angiogenesis. Oral delivery of medication is the holy grail of the drug industry, and Dr. Ferrari's group is developing microtechnology that delivers therapeutics via oral ingestion. The pill containing the material is transported through the gastrointestinal tract and released in the gut wall. He reported that this is a potential delivery mechanism for peptides and microfabricated transmucosal patches.

Panel Discussion

During the panel discussion, Dr. Desai (University of Chicago) noted that it is especially important to determine whether the new nanodevices are more effective and less expensive than the current technologies being used. Dr. Baker stated that if real-time monitoring were incorporated into nano devices, it would allow clinicians to follow individuals and detect when normal tissue becomes abnormal or premalignant and progresses to malignancy. If predisposition can be defined along with the line between malignancy and normal cells, it will clarify the pathophysiology and may lead to effective interventions.

In response to Dr. Whiteside's question of whether it would be practical or beneficial to screen every individual, Dr. Baker noted that this is already occurring in a sense, through the use of high-resolution computed tomography and mammography. These techniques are identifying lesions whose significance is unclear. Dr. Baker added that his research group is not trying to kill large tumors with their device because these most likely would be removed surgically; rather, they are targeting diseased cells in small tumors or precancerous lesions.

During the discussion, the speakers also talked about the problems in application of their technologies. These included problems in reaching out and interacting with biologists and the clinical community, technological hurdles, a need for a common language between scientists in different fields, collaboration, and funding obstacles.

Breakout Sessions

Drs. Lee Hood (Institute for Systems Biology) and Sudhir Srivastava (NCI) moderated deliberations in the breakout session on nanotechnology platform for high-throughput identification of biomarkers. The focus of the breakout session was to identify what might be done in the short-term and the long-term to leverage advances in nanotechnology for high throughput identification of biomarkers.

Both NIST and the NCI are trying to identify nanotechnology research that could be funded and determine how these agencies could serve as leaders in this area. The focus of the discussion was high throughput analysis, multiparameter analysis, and gene expression patterns.

Multiparameter analysis is needed for cancer diagnosis, treatment, and prevention. Diagnostic targets include fluids (blood) and cells (blood, biopsy, and in vivo). It was agreed that the ability to profile proteins in blood would be very helpful, and nanotechnology offers insights into the ability to use a few cells from a biopsy sample. It was felt that gene expression patterns would be an important area for application. Similarly, macrophages could be used as remote biodetectors for inflammation, neoplasia, and other aberrations in the human body.

The discussion touched on the problem of the dynamic range of protein expression (10^6 to 10^7). Nanotechnology could lead to techniques that can be miniaturized with parallel functioning. It was speculated that shortly, researchers would be able to evaluate 1 million proteins per day through protein chips with chromatographic surfaces. Biomarker pattern recognition software is being developed with multiparametric analysis capability for high selectivity and specificity. The need to standardize the statistical and analytical approaches was identified. Nanotechnology could help in detection of modified proteins at the nano level.

A high throughput discovery process is needed to investigate modified proteins as cancer biomarkers. Nano-barcodes also could be useful for biomarker discovery. This nanoscale assay technology allows analysis of bioanalytical data on cells, proteins, and low-molecular-weight organics present in very small sample volumes. The assay for the nano-barcodes could be the matrix-assisted laser desorption/ionization (MALDI) technique, a chromatographic resin, or a fluorescent marker. This technology offers a solution-based array that gives high throughput and can perform analysis at RNA and DNA levels, while avoiding many of the problems associated with chip format. The addresses of 200 to 300 bar codes are read efficiently by imaging particles as they pass by. It could identify hundreds of thousands of codes simultaneously.

It was agreed that moving microfluidics/microelectronics into the nano scale would be one of the most exciting areas for cancer diagnosis, prevention, and treatment. It was concluded that most nanotechnologies do not have the throughput that is needed and have to be developed.

In a separate, concurrent breakout session, Dr. George Whitesides moderated deliberations on nanotechnology devices for early detection of cancer. The focus of this breakout session was nano-devices and data acquisition for identification of biomolecules. Discussions indicated that nanotechnology could complement advances in molecular biology and could potentially reduce the noise level associated with proteomic and genomic analyses. Successful analytical techniques developed using nanotechnology may not require amplification, thereby preventing the accompanying distortion. For example, these techniques may eliminate the need for PCR analyses in some instances.

The group felt that the significant problems in measuring multiple species at small concentrations and moving to a small-volume scale could be overcome with advances in nanotechnology, which would also allow for the direct measurement of very small quantities. Discussions emphasized that nanodevices would need high-throughput capabilities and the future development should address the tradeoff between speed and noise. This rapidly developing science also may provide new kinds of information not available through genomics and current molecular screening techniques.

Discussions also touched on the ability to detect molecular and cellular effects in a selective and sensitive manner such as room temperature quantum effects. Hierarchy of information should be thoughtfully considered when utilizing nanotechnology. Nanotechnology can be used to enhance the accuracy of existing diagnostic methods, thereby providing a new window into the cell. It also can provide information on cells without damaging or removing them from their environment.

Dr. Whiteside's session cautioned that development of nanodevices needs to proceed in the context of existing technologies to minimize duplication of efforts. Nanodevices hold promise for conferring the ability to examine multiple activities in molecules and/or cells over time and at the same time, which is important in understanding the evolution of cells.

From the systems perspective, nanodevices could be used to determine the pathway of malignancy. Nanotechnology has the advantage of multiplexing at the cellular level and may shed greater light on the life cycle of the normal cell and the point at which molecular processes and changes within cells become correlated with the development of cancer. It should be possible to develop high-density arrays utilizing nanotechnology and to obtain a very large amount of information from a small source.

One of the major challenges recognized by groups developing this technology was finding ways to bring physics and engineering communities together with biologists and clinicians in a productive, synergistic and complementary manner.

Recommendations

After a day and a half of deliberations, the workshop participants presented their recommendations for the implications of nanotechnology in early detection of cancer.

(1) Nanotechnology has the potential to make significant contributions to cancer prevention, detection, diagnosis, and treatment. Tools are important and integral parts for early detection, and nanotechnology can provide novel tools and complement existing ones. This technology offers opportunities in multiple platforms for parallel applications, miniaturization, integration, and automation.

(2) Nanotechnology could be extremely useful in the area of biomarker research. It could provide successful strategies for real time and direct readout of

genomic and proteomic information at the single-molecule and single-cell level. Additionally, it would allow for multiparametric analysis using relatively small sample volumes. Nanotechnology could help provide additional sensitivity in assays through analysis of single cells and extremely limiting amounts of samples.

(3) Four specific nanotechnology applications that could impact on biomarker research for early detection are as follows: (a) nanostructures such as pores, (b) nanoprobes such as scanning tunnel microscopy, (c) nanosources such as laser-induced fluorescence, and (d) nanomaterials such as superparamagnetics and quantum dots.

(4) Nanotechnology could have a profound influence on cancer prevention efforts because it offers innovative tools for understanding the cell as well as the differences between normal and abnormal cells. This technology could provide insights into the mechanism of transformation, which is fundamental in designing preventive strategies. Further, it provides novel observation modalities, which are nondamaging, into the cellular machinery. It allows for the analysis of such parameters as cellular mechanics, morphology, and cytoskeleton, which has been hard to achieve using conventional technology.

(5) The participants further recommended that cross-cultural exchanges among the physical, biological, engineering, and medical communities be encouraged, facilitated, and promoted. Such fostering of interdisciplinary fields will enhance the translation of nanotechnology applications into biological realities for early detection and prevention of cancer.