Next Generation Nanosolutions for Cancer Treatment and Diagnosis

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Abstract

This review describes several state of the art advances made in the field of cancer nanotechnology that bring the science closer to clinical realization for application in disease treatment and diagnosis. For therapeutic delivery, *in vivo* strategies to improve the biodistribution, tumor penetration, and cellular uptake of nanocarriers are discussed, including the precise control of formulation and conjugation of targeting agents. We highlight recently developed novel nanosolutions that specifically address cancer metastasis. We also describe in detail the promising use of nanotechnologies for *in vitro* diagnostics on tissue section samples, an area that appears to be ready for clinical application in the near future. Finally, we discuss emerging discoveries on the unique biophysical properties of cancer that hold promise for paradigm shifts in future cancer diagnosis and treatment strategies as this field continues to mature. (Cancer & Chemotherapy Rev. 2008;3:144-51)

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Key issues

- *Development of strategies to enable nanocarriers to bypass liver and spleen accumulation is central to achieving cancer targeting.*
- *We do not yet have nanocarriers that show great tumor penetration in vivo.*
- *Surface-enhanced Raman spectroscopy, QD-based IHC, and QD-based ISH of tissue sections are three nanotechnology-based approaches that show tremendous promise for multiplex biomarker quantitation in routine clinical diagnostics.*
- *Promising biophysical property markers of cancer that do not rely on functionalization with targeting agents will need to be explored to realize the full potential of nanotechnology in cancer diagnosis and treatment.*

Introduction

Given its relatively recent introduction and application in the field of cancer, the majority of current nanotechnology platforms for chemotherapy have involved repackaging traditional anticancer agents into various forms of nanometer-sized delivery vehicles, such as monomeric polymer-drug conjugates with sizes generally 10 nm or less[1], polymeric nanoparticles[2] or self-assembled amphiphilic block-copolymer micelles[3] in a range of 20 and 100 nm, or lipid-[4] or polymeric vesicles[5] (also called liposomes and polymersomes, respectively) with size sub-100 nm to sub-micrometers. As for diagnostics, advances in micro- and nanofluidics have enabled high-throughput discovery of a growing list of putative or candidate biomarkers that have yet to be translated for application in the clinical setting[6-9]. Such platforms arguably represent only an incremental advancement when considering the vast possibilities for unconventional strategies if the length-scale of nanotechnology were exploited to its fullest potential.

The development of first generation anticancer nanocarriers has been focused on the formulation of delivery vehicles using well-developed biomaterials and formulation methodologies noted above, and on targeting and treatment of primary tumors based mainly on Enhanced Permeation and Retention effect, which refers to the accumulation of nanoparticles in tumor facilitated by the highly permeable nature of the tumor vasculature and poor lymphatic drainage of the interstitial fluid surrounding a tumor[10]. The second generation nanocarriers described in this review, however, place a greater emphasis on novel strategies (i) to bypass biological barriers at the systemic, tissue, and cellular levels, and (ii) to locate and target metastatic lesions. New chemistries and fabrication technologies now allow unprecedented, precise control of nanocarrier formulation, making it possible to evaluate nanocarriers with the variation of one parameter (e.g. size, surface property, and shape) at a time to provide insight into the fundamental understanding of the interplay of these parameters and the *in vivo* performance of the nanocarriers.

With respect to medical diagnostics, we discuss techniques using nanoparticles that have real potential to be adopted and used in the near future in clinical and pathology laboratories to generate information on tumor classification, stage, and grade from surgical and biopsy samples. In particular, we focus on the advances that enable direct application of these nanoparticles onto tissue sections, since the ability to interrogate prognostic and predictive molecular biomarkers in the context of morphology would be invaluable for molecular histopathology and clinical diagnostics. Finally, innovative strategies to determine metastatic potential based on biophysical cellular properties are discussed to introduce a novel approach for prognostic classification of cancer.

New Chemistries and Fabrication Techniques

The formulations of nanocarriers with well-controlled properties (e.g. size, surface characteristics) and in large quantities are essential to their clinical translation. Several new conjugation chemistries and fabrication techniques have shown some promise for controlled formulation of nanocarriers. "Click chemistry," a reaction conceived by Nobel laureate K. Barry Sharpless, refers to "clicking" together several specific functional groups covalently, and it allows conjugation of therapeutic agents and targeting ligands to nanocarriers with unprecedented site-specificity[11, 12]. The "click" process involves 1,3-dipolar cycloaddition of an azide and an alkyne to form 1,2,3-triazole, a reaction known for its high efficiency, high specificity, and solvent tolerability. Click chemistry proceeds well in aqueous solution-[13]or even in live organisms[14, 15], and is independent of other functional groups[11]. One unmet challenge in *in vivo* tumor targeting is the production of antibody-conjugated nanocarriers in a highly specific and reproducible manner. Coupling chemistry will

never lead to controlled conjugation of antibody to nanocarrier surface with the anticipated specificity. However, click chemistry may provide a solution. If an azide group by means of an azide-containing nano-natural amino acid is incorporated into the antibody, which is technically attainable via protein bioengineering[16], the site-specific conjugation of the azide-containing antibody to alkyne-containing nanocarrier could be easily achieved.

Polymeric nanoparticles, which are one of the most widely used platforms in drug delivery, are usually prepared by co-precipitation of hydrophobic polymers with the drug. However, this formulation method often leads to formation of nanoparticles with poorly controlled physicochemical properties, such as low drug loading, uncontrolled drug release kinetics, heterogeneous nanoparticles, and broad particle size distribution[2]. To address these challenges, Cheng and Tong developed a drug-incorporation strategy by using Zn-paclitaxel as the catalyst to mediate controlled polymerization of lactide, thus allowing quantitative incorporation of paclitaxel to polylactide, a biocompatible polymer[17]. When bulky chelating complex is used, the Zn-catalyst regulates the initiation and polymerization via the least sterically hindered hydroxyl group of paclitaxel, which results in paclitaxel-polylactide conjugates with precisely controlled composition and molecular weights. At low monomer/initiator (lactide/paclitaxel) ratio, the drug loading of paclitaxel-polylactide and the nanoparticle derived from the conjugates have extremely high loadings (close to 40%) and controlled-release kinetics.

Bottom-up formulation strategy usually gives rise to nanostructures with relatively broad particle size distributions and almost exclusively spherical shape. A top-down nanofabrication technique called particle replication in non-wetting templates (PRINT), developed by DeSimone and his team, addressed these limitations and allowed large-scale formulation of polymeric nanoparticles with precisely controlled sizes and shapes (e.g. cylinder, cube, disc) using soft lithographic molding technology[18,19]. They used photocurable perfluoropolyether molds to emboss liquid precursor compounds using highly fluorinated surfaces that are non-wetting to organic materials, which enables the fabrication of isolated objects with superior shape and composition control[19].

Nanocarriers for Chemotherapy

Properties Affecting Biodistribution and Intratumoral Penetration

To achieve tumor targeting, nanocarriers must first overcome systemic barriers, especially clearance via phagocytic uptake and hepatic filtration. Then they are expected to extravasate the tumor vasculature and penetrate the tumor microenvironment, so that even the cancer cells situated distal to the tumor vessel could be exposed to the anticancer agent at high enough concentrations. Here we describe the most recent findings on the physical properties of nanoparticles that affect biodistribution and intratumoral penetration, as well as some potential strategies for designing them for optimal biodistribution.

It is well known that nanoparticle size, surface functionality, and charge affect biodistribution. Particles with size 70-200 nm seem to be ideal for cancer treatment. With regard to biodistribution, large particles (> 200 nm) tend to induce response by the reticuloendothelial system and are thus quickly cleared by immune system. Particles ≤ 150 nm can escape through fenestration of the vascular endothelium and get cleared from the circulation, and particles < 20-30 nm are easily cleared through the kidney or lymph nodes[20, 21]. Thus, it is unclear which size range is ideal for prolonged circulation half-life, although there is a general consensus that particle size should be controlled to be < 200 nm[21].

Penetration of the intravascularly administered nanocarriers or even small molecule chemotherapies into the tumor mass has been proven difficult because of the high interstitial fluid pressure and complex extracellular cellular matrix of the tumor tissue[22]. Chilkoti, et al. evaluated dextran delivery vehicles and demonstrated a molecular weight (size)-dependency of their tumor penetration[23]. Dextrans of 3.3-10 kDa penetrated deeply and homogeneously into the tumor tissue from the vessel wall, whereas a high concentration was observed only approximately 15 μm from the vessel wall for 40-70 kDa dextran. Using a three-dimensional, multicellular spheroid of SiHa (human cervical carcinoma) cells that simulates a solid tumor, Pun, et al. observed a similar size-dependency of nanoparticles on tumor penetration[24]. Polystyrene nanoparticles with 20 or 40 nm sizes readily penetrated this simulated tumor and distributed homogeneously**,** whereas 100 and 200 nm particles showed restricted penetration. Interestingly, when extracellular matrix-disrupting collagenase was coated on the nanoparticle surface, roughly 10-fold enhancement of tumor penetration for the 20 and 40 nm particles was observed[24]. This study provides insight into a strategy that could potentially be employed for enhancing tumor penetration.

Geng, et al. demonstrated for the first time that the shape of delivery vehicles also has a significant effect on biodistribution[25]. They evaluated cylinder-shaped filomicelles (20-60 nm in cross-sectional diameter and a few micrometers in length) in rodents and found that the filomicelles could persist in the circulation up to one week after intravenous

injection, which is about ten-times longer than their spherical counterparts and is more persistent than any known synthetic nanoparticle. The enhanced circulation of filomicelles is presumably because the cylinder-shaped delivery vehicles are more readily extended by flow and, therefore, are less likely to interact with and get taken up by phagocytic cells. This interesting finding may shed light on the design of a new generation of drug delivery system for enhanced circulation and improved *in vivo* performance. DeSimone, et al. prepared polymeric nanoparticles of various shapes (e.g. cylinder and cube) using the aforementioned PRINT technique and demonstrated *in vitro* that shape greatly impacted the cellular uptake of nanoparticles[26]. Cylindrical nanoparticles with an aspect ratio (height/width) of 3, for example, can be taken up by cells four-times faster than cylindrical shaped nanoparticles with an aspect ratio of 2. It has yet to be determined whether these uniquely designed nanoparticles could outperform the traditional, spherical nanoparticles in biodistribution and antitumor efficacy studies.

Besides size and shape, the surface characteristics and physical properties of nanocarriers are well known to influence nanoparticle biodistribution. Positively charged particles typically are cleared much more quickly from the circulation than neutral or negatively charged particles[27]. The use of polyethylene glycol (PEG) to modify the surface of nanoparticles is critical for improving circulation half-life and reducing plasma protein absorption to nanoparticles that could otherwise lead to opsonization (a process that involves surface deposition of blood opsonic factors, such as fibronectin, for enhanced recognition by macrophages)[27]. There has been some progress for developing new PEG-like, protein-resistant materials. One interesting new material is zwitterionic polymers that exhibit high resistance to nonspecific protein absorption due in part to its neutral surface charge and hydrophilicity[28], but it is unclear at this time whether this could be a viable, biocompatible alternative to PEG.

There are various pathways for cellular uptake of nanoparticles, such as receptor-mediated endocytosis and TAT-peptide-mediated cell penetration, which involves passage through the cell membrane by generating a transient hole. Recently, Verma, et al. discovered that gold nanoparticles coated with sub-nanometer striations of alternating anionic (sulfonate) and hydrophobic (methyl) groups can successfully penetrate plasma membrane without bilayer disruption and could be particularly useful for direct delivery of cargos to the cytoplasm[29]. Interestingly, this nanoparticle also showed excellent resistance against protein absorption, potentially providing another strategy for surface coating in novel formulation of nanoparticles.

Aptamer-Mediated Tumor Targeting

Tumor targeting has been extensively evaluated using traditional targeting ligands such as small molecules, peptides, and proteins. Aptamers, single-stranded DNA, RNA, or oligonucleotides that can fold into unique conformations capable of binding to specific targets with high affinity and specificity, recently emerged as a new class of targeting ligands that showed some uniqueness unattainable from antibody or small molecules[30, 31]. Farokhzad, et al. demonstrated for the first time that intratumorally administered polymeric nanoparticles with surface-coated aptamers specific for prostate-specific membrane antigen (PSMA) could successfully recognize and target PSMA-positive lymph node carcinoma of prostate (LNCaP) cells and eradicate the tumor more effectively than the nanoparticles without aptamers[32]. When injected systemically, these nanoparticle-aptamer conjugates could target subcutaneously implanted LNCaP tumor[33], and the *in vivo* targeting efficiency correlated well with the surface density of the aptamer ligands[34]. Like many other nanoparticulate delivery vehicles, aptamer-conjugated nanoparticles are still subject to hepatic and splenic accumulation, which is comparable to the untreated nanoparticles[33,34]. In fact, poor biodistribution owing to enhanced reticuloendothelial response remains a challenge for numerous multifunctional nanoparticles containing enabling ligands on nanoparticle surface.

Antimetastatic Nanotherapies

Now we highlight some recent nano-enhanced strategies for tackling disease dissemination, which is especially significant as a patient's prognosis declines sharply with the onset of metastasis. Tumors usually contain a meshwork of clotted plasma proteins in the tumor stroma and vascular walls, but no such meshwork is found in normal tissues. Simberg, et al. developed self-accumulating nanoparticles by conjugating a novel peptide sequence (CREKA, a pentapeptide selected by phage display that can target the clotted plasma protein) onto the surface of 50 nm super-paramagnetic iron oxide nanoparticles[35]. Accumulation of these nanoparticles in tumor stroma can induce additional local clotting and, thereby, attract more CREKA-coated iron oxide nanoparticles, resembling to some extent the role platelets play in wound healing. The investigators envision that such accumulation could be used to (i) physically disrupt vasculature at the primary tumor site to prevent metastasis as well as (ii) visually enhance magnetic resonance contrast via increased concentration of iron oxide nanoparticles to better diagnose disease.

Benny, et al. showed that by conjugating TNP-470, an analog of the antiangiogenic fumagillin discovered by Judah Folkman, to monomethoxy-polyethylene glycol-polylactic acid to form polymeric micelles, its bioavailability was increased significantly to allow oral administeration[36]. The team found this nanocarrier to be especially efficacious in preventing the development of liver metastasis in mice. Researchers in the laboratory of Cheresh, who discovered the presence and role of $\alpha_v\beta_3$ integrin in angiogenesis, recently reported a targeted nanotherapeutic that had modest effect on the primary tumor but was highly effective in preventing disease dissemination[37]. By encapsulating doxorubicin in liposome coated with arginine-glycine-aspartic acid (RGD)-targeting ligand (RGD serves as the recognition site for integrin receptors), the team was able to increase the anti-metastatic activity of doxorubicin by 15-fold and effectively prevent metastasis in their animal models of pancreatic and kidney cancers. While these nanoplatforms described require additional optimization to facilitate their clinical utility, they open the door for developing future nanotechnology devices tackling cancer metastasis.

Nanotechnology for Clinical Diagnostics

Presently, the most commonly used method for analyzing protein expression on tissue is immunohistochemistry (IHC), where the detection of protein analytes is accomplished through a multistep process of first binding specific primary antibodies to the analytes, then detecting this antigen-antibody complex via a secondary detection antibody labeled with a reporter tag, such as a fluorescent dye, enzyme, or radioactive compound. Immunohistochemistry is not only fraught with sensitivity and interobserver reliability problems, but IHC using traditional, organic dyes also faces severe limitations for multiplexing, largely due to the overlap of fluorescence signals that have broad emission peak width. Multiplex tissue analysis, where multiple analytes are detected simultaneously, would allow us to examine co-expression and spatial distribution of several proteins, and the nanotechnologies described below make that possible.

Surface-Enhanced Raman Scattering Nanoparticles

One such nanotechnology whose success on human tissue samples has just recently been demonstrated involves the use of Raman scattering-based nanoparticles. Raman scattering refers to inelastic scattering of monochromatic light (from a laser source) that is used to study the vibrational and rotational modes in the atomic lattice of a solid. When the light hits a solid material, the energies of the photons in the light are shifted up or down and generate the Raman spectrum. Normally, Raman intensity is very low compared to fluorescence intensity, but its signal intensity can be greatly enhanced (10^6-10^{14}-fold) to enable the detection of a single molecule by adsorbing Raman-active molecules onto the nanoparticle surface, known as surface-enhanced Raman scattering (SERS). Raman nanoparticles can be made of gold nanoparticles with Raman-active molecules and then coated with silica for stabilization (i.e. to prevent disintegration of nanoparticles) and functionalized by attachment of biomolecules such as antibodies[38]. They can also be made of an aggregate of silver nanoparticles with Raman-active molecules, such as basic fuschin, rhodamine 6G, acridine orange[39].

Last year, Sun, et al. demonstrated the first successful application of Raman spectroscopy by performing multiplex protein assay (for prostate specific antigen (PSA) and cytokeratin-18) directly on formalin-fixed, paraffin-embedded (FFPE) tissue sections of the human prostate, and generating tissue imaging data[40]. Being able to demonstrate the feasibility of multispectral staining on FFPE samples was important because the vast majority of clinical materials are routinely processed and archived in the form of FFPE blocks. Their nanoparticles, also called composite organic-inorganic nanoparticles (COIN), were comprised of clusters of silver nanoparticles plus Raman-active molecules encapsulated in cross-linked bovine serum albumin for particle stabilization, functionalization, and surface enhancement of Raman scattering. To distinguish the Raman spectra of individual COIN specific for different analytes, the multiplex spectra comprised of signals from the two types of COIN as well as the signal arising from tissue autofluorescence and other nonspecific, background noise were de-convoluted by least-squares regression. Spectra were collected throughout defined points in a raster scan with each spot on the tissue being denoted as PSA-positive or PSA-negative to generate a map of PSA expression in tissue sections.

Compared to the traditional fluorescent dyes that have broad emission bands (> 50 nm), the emission band of Raman nanoparticles is much narrower (< 2 nm). In addition, a greater number of unique optical "signatures" could be developed by varying the structures of the embedded Raman-active molecules, and these characteristics make them well-suited for multiplexing. Other advantages associated with Raman nanoparticles include

resistance to photo-bleaching, and easy distinction of the Raman signal from the background autofluorescence signal inherent in tissue samples. Most importantly, because these COIN nanoparticles are conjugated to primary antibodies, the multiple sequential staining steps that are necessary for most IHC assays can be eliminated. Despite the fact that spectral intensity is proportional to analyte concentration, quantitation of protein expression is yet to be demonstrated for this technology.

Quantum Dot-Based Multiplex Immunohistochemistry in Tissue

Myriad advances have been made in the development of quantum dot (QD)-based technologies, but clinical application of these semiconductor nanocrystals with size-dependent fluorescence emission as labels for IHC and tissue analysis has not yet achieved much success or widespread adoption. In 2006, Fountaine, et al. were the first to demonstrate success in using five different QD probes simultaneously to perform multispectral imaging of proteins on FFPE tissue sections to analyze the morphologic characteristics of human lymphoid tissue[41]. Confocal laser scanning microscopy was used to detect the five different signals from streptavidin-conjugated QD that could bind to biotinylated secondary antibodies. In order to achieve multiplexed stains, the authors noted that it was critical to add an avidin-biotin blocking step in between the application of the QD and the next set of primary antibodies in this process involving repeated, sequential binding of five sets of primary antibodies to five different surface biomarkers for various lymphoid immune cells.

Since QD reportedly have signal brightness that surpasses that of traditional, organic fluorophores, these researchers tested whether QD conjugated to secondary antibodies could be effectively used in tissue imaging. The results were disappointing, with QD-conjugated secondary antibodies giving a weak signal even though the primary antibody chosen was against CD20, which is expressed at high levels on the cell surface. Another limitation they encountered was that when the confocal microscope and detector were configured for detection of five QD signals, the signal overlap between QD within 20 nm of each other could not be resolved and became problematic (e.g. between QD 565 nm and QD 585 nm).

More recently, another group of researchers led by Shuming Nie has also successfully demonstrated the use of up to five different types of QD for multiplexed and quantitative IHC on FFPE tissue[42]. While Fountaine, et al. could not get the QD-secondary antibody conjugates to work very well in generating high-intensity fluorescence, Xing, et al. were successful in using QD-secondary antibody conjugates recognizing different sets of primary antibodies raised from different animal species[42]. However, Xing, et al. did acknowledge that direct staining with QD conjugated to primary antibodies, which would allow staining with several different types of QD in just a single step, is not always possible. The authors explained that some primary antibodies might not survive the QD conjugation process, and their binding properties could be altered by covalent modifications at either $-NH_2$ or -COOH sites. A related concern that has been expressed by many investigators is that QD can only be used for proteins expressed at high levels, but interesting cancer biomarkers may be present at low concentrations or only in a small number of cells.

In order to perform quantitative analysis of biomarker expression, Xing, et al. developed an integrated image processing and bioinformatics software tool called Q-IHC[42]. Quantification of biomarker features into numerical values by Q-IHC involved ''ratiometric'' staining, in which one of the QD-antibody conjugates targeted a housekeeping gene product and provided an internal standard for signal calibration and quantification. These researchers validated their QD staining data by comparison to the standard pathology protocols, in which slides from FFPE tissue blocks were stained for three breast cancer biomarkers: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor type-2 (HER2). The traditional IHC results were scored from 0 (no visible staining in the nucleus or membrane) to 3+ (strong and complete membrane or nuclear staining in more than 10% of malignant stained cells). The results revealed that a 3+ score for ER, PR, or HER2 by traditional IHC corresponded to 85-100% relative expression of the antigen (as determined by QD quantitation) and that 1+ or 2+ scores corresponded to 11-48% expression. Classification of antigens expressed at low levels (1+ or 2+) was subjective and often resulted in considerable interobserver variability. In contrast, quantitative QD measurements allowed accurate determination of tumor antigens at low levels.

It should be noted that the attempts of other researchers in trying to get QD to work in QD-based multicolor and quantitative imaging have not been met with much success. One of the reasons for this, as explained by Xing, et al., could be that these size-tunable QD (the fluorescence emission spectra of ZnS-capped CdSe QD can be tuned from blue to red by changing the core particle diameter from 1.5 to 6.0 nm) show considerable variation in signal brightness at different emission colors[42]. For example, the integrated signal intensity of green QD (525 nm emission) is 17-times lower than that of red QD (655 nm emission) and almost 32-times

lower than that of near-infrared QD (705 nm emission) under identical experimental conditions. The authors stated that when these QD are used to quantify biomarker expressions in the same cells or tissue specimens, the results could be misleading.

Quantum Dot-Based Multiplex Immunohistochemistry in Tissue

As research on gene expression profiling and gene signatures for predicting clinical outcome or response to therapy identifies an increasing number of candidate nucleic acid biomarkers, there is a real need for enabling the detection of these signatures in association with particular cell types and lineages, and spatial localization in tissue samples. More importantly, a major challenge and limitation of IHC methods is that specific antibodies against many of the protein analytes of interest are not available. Working with mRNA targets would bypass this dependence on antibodies because the oligonucleotide probes used in colorimetric RNA *in situ* hybridization (ISH), which is the current standard methodology for detecting gene expression in tissue, can be constructed easily for any gene.

Until now, there have been relatively few reports of QD use for ISH, but researchers in the laboratories of Richard Byers and Massimo Loda have recently demonstrated great success in detecting gene signatures in tumor tissue by semi-automated, QD-based ISH (Q-ISH)[43, 44]. They were able to demonstrate the feasibility of (i) QD conjugation to oligonucleotide (50-mer) cDNA probes, which was a technical hurdle, (ii) the use of QD-labeled oligonucleotide probes in high-throughput, automated ISH on FFPE tissue, followed by (iii) spectral imaging and signal quantitation using a fluorescence microscope and CRI Nuance spectral analyzer (CRI Inc., Woburn, MA). The fact that QD-based fluorescence has a linear relationship between the amount of probe hybridized and signal intensity, which is not seen with chromogenic methods, was an essential feature for enabling accurate measurement of relative transcript levels in the tissue.

Byers, et al. applied Q-ISH to analyze the expression of transcription factors ASCL1 and the homeobox-containing gene NKX2-2, which had been reported as members of a gene-expression signature set associated with poor prognosis in malignant gliomas[43]. The analysis was performed on two different human glioma tissues, and showed abundant expression of both markers in the majority of glioma cells with absent or low-level expression in the normal white matter adjacent to the tumor; these results were similar to those observed by IHC. This demonstrated how Q-ISH technique could help facilitate the clinical translation of gene-expression signature discoveries.

Future Perspectives

As the field of cancer nanotechnology further matures with an increasing number of nanotechnologies moving closer to clinical application, there is room for continued efforts in developing the next-generation nanosolutions for the prevention of disease progression and dissemination, and for diagnostics that do not solely rely on biomarker identification via antibodies. We are now witnessing biophysics and cell mechanics emerging as areas of study with tremendous potential for application in cancer. For example, the environment surrounding solid tumors has components that confer, in the macro scale, a distinct viscoelastic property ("squishiness"), which the clinicians have traditionally exploited when manually palpating for the presence of disease. Studies have demonstrated that global alterations in cell mechanics at the micro/nanoscale play an integral role in cancer progression. Paszek, et al. demonstrated that by changing the rigidity of the three-dimensional culture matrix (representing an *in vitro* breast cancer model), the malignant phenotype could be repressed or enhanced[45]. This was heavily dependent on regulators of cytoskeleton rearrangement Rho-guanosine triphosphatase, specifically the downstream effector Rho-kinase (ROCK)[45]. The authors postulate that tumorigenic behavior may be triggered as a response to biophysical rather than only a biochemical cue in the microenvironment. This is not entirely surprising given that biological barriers encountered by metastatic cells, such as when negotiating extracellular matrices, invading surrounding tissues, and traversing in/out of vasculature and lymphatics[46], are all associated with significant changes in physical forces and stresses. Indeed, chemical inhibition of ROCK has been shown to be effective in preventing dissemination of malignant brain tumors[47]. Recent studies have demonstrated such inhibition causes dramatic biophysical alterations in cell mechanics, where particle-tracking measurements of nanoparticles imbedded in cells, termed micro/nanorheology, revealed a 25-fold change in viscoelastic properties caused by ROCK inhibition[48].

Earlier this year, researchers at UCLA reported preliminary results of detectable difference in cancer cell mechanics using samples harvested from patients with lung, breast, and pancreatic cancer[49]. The team performed nanomechanical studies to probe the cell mechanics of different cancer types by depressing the cell using an atomic force microscope tip. The investigators found that different cancer types had unique viscoelastic properties and that metastatic cells distinguished themselves from their non-metastatic counterparts by being more than 70% softer. Exploiting the nanoscale to probe and detect this and other non-traditional (i.e. thermo-

dynamic, electrical) changes in cellular processes involved in cancer progression may yield paradigm-shifting nanosolutions for cancer treatment and diagnosis. Indeed, the National Cancer Institute has already begun to gather experts from the physical sciences to discuss how to efficiently integrate such ideas for future cancer applications.

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