

Biomedical Nanotechnology With Bioinformatics—The Promise and Current Progress

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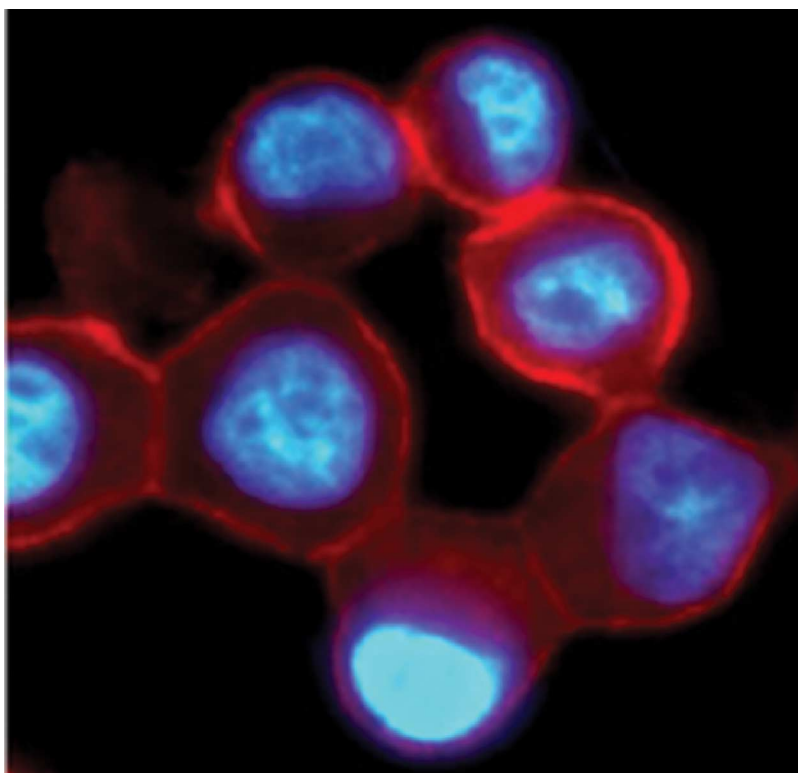
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Integrating Biology, Nanotechnology, and Informatics will speed up the translation of results from bench to bedside for personalized medicine. It is a cross-disciplinary research that combines science, engineering, and medicine. In particular, biomedical nanotechnology has broad applications for molecular imaging, molecular diagnosis, and targeted therapy. The basic rationale is that nanometer-sized particles such as semiconductor

quantum dots and iron oxide nanocrystals have optical, magnetic or structural properties that are not available from either molecules or bulk solids. When linked with biotargeting ligands such as monoclonal antibodies, peptides or small molecules, these nanoparticles can be used to target diseased cells and organs (such as malignant tumors and cardiovascular plaques) with high affinity and specificity. In the “mesoscopic” size range of 5–100 nm diameter, nanoparticles also have large surface areas and functional groups for conjugating to multiple diagnostic (e.g., optical, radioisotopic, or magnetic) and therapeutic (e.g., anticancer) agents. The capacity and potential of nanoparticles to perform multiple functions simultaneously in medical treatment is a new dimension for clinical applications. Recent advances have led to multifunctional nanoparticle probes for molecular and cellular imaging, nanoparticle drugs for targeted therapy, and integrated nanodevices for early disease detection and screening. These developments have opened exciting opportunities for personalized medicine in which disease detection, diagnosis, and therapy are tailored to each individual’s molecular profile, and also for predictive medicine in which genetic/molecular information

is used to predict disease development, progression, and clinical outcome.

For medical applications, nanotechnology is often linked with biomolecular signatures or biomarkers that are correlated with a biological behavior or a clinical outcome. These markers are commonly defined as mutant genes, RNA, proteins, lipids, carbohydrates, small metabolite molecules, and altered expression of them. The high throughput data of these markers can be quantified with patterns by computation. For individualized therapy, biomarkers enable the characterization of patient populations and quantification of the extent to which new drugs reach their intended targets. One example is the drug trastuzumab (Herceptin; Genentech/Roche), a monoclonal antibody designed to target amplified and overexpressed *ERBB2* (also known as HER2) tyrosine kinase receptor found in only ~30% of breast cancers (see the photograph). In this “Point of View” article, we briefly discuss the current status of biomedical nanotechnology, the linking with computation, and its promise in personalized medicine.

Quantum Dot Nanotyping: A prototype nanotechnology is semiconductor quantum dots, which are tiny light-emitting particles on the nanometer scale and are under intense development as a new class of fluorescent probes for molecular imaging and medical diagnostics. In comparison with organic dyes and fluorescent proteins, quantum dots have unique optical and electronic properties such as size-tunable light emission, superior signal brightness, resistance to photobleaching, and simultaneous excitation of multiple fluorescence colors. These “programmable” properties are most promising for improving the sensitivity and the multiplexing capabilities of molecular histopathology and disease diagnosis. Recent research has led to highly bright and stable QD probes that are well suited for profiling genetic and protein biomarkers in intact cells and clinical tissue specimens. In contrast to *in vivo* imaging applications where

the potential toxicity of quantum dots is a major concern, immunohistological studies are performed on *in vitro* or *ex vivo* clinical patient samples. The use of multicolor QD probes in immunohistochemistry (IHC) is one of the most important and clinically relevant applications in the near term. At the present, however, clinical applications of QD-based immunohistochemistry are still early in their development and have achieved only limited success. A major bottleneck is the lack of robust protocols and experimental procedures that define the key factors and steps for quantum dot immunohistochemistry. In particular, there are currently no consensuses on methods for QD-antibody bioconjugation, tissue specimen preparation, multicolor QD staining, image processing, or data quantification. In a recent *Nature Protocols* (Vol. 2, No. 5, pp. 1153–1165, 2007) article, Wang and coworkers have started to address these issues by combining the nanotechnology with quantitative computation. They have demonstrated that bioconjugated quantum dots can be used for multiplexed profiling of molecular biomarkers, and ultimately for correlation with disease progression and response to therapy.

In Vivo Tumor Imaging: Traditional *in vivo* imaging probes or contrast agents include radioactive small molecules in positron emission tomography (PET) and single photon emission computed tomography (SPECT), gadolinium compounds in magnetic resonance imaging (MRI), and isotope-tagged antibodies. In comparison, bioconjugated QDs and targeted nanoparticles provide a number of unique features and capabilities that could significantly improve the sensitivity and specificity of disease imaging and diagnosis. First, the size-dependent optical and electronic properties of QDs can be tuned continuously by changing the particle size. This “size effect” permits the use of a broad range of nanoparticles for simultaneous detection of multiple cancer biomarkers. Second, nano-

particles have more surface area to accommodate a large number or different types of functional groups that can be linked with multiple diagnostic (e.g., radioisotopic or magnetic) and therapeutic (e.g., anticancer) agents. This opens the opportunity to design multifunctional “smart” nanoparticles for multi-modality imaging as well as for integrated imaging and therapy. Third, extensive research has shown that nanoparticles in the size range of 10–100 nm are accumulated preferentially at tumor sites through an effect called enhanced permeability and retention (EPR). This effect is believed to arise from two factors: 1) growing tumors produce vascular endothelial growth factors (VEGF) that promotes angiogenesis; and 2) many tumors lack an effective lymphatic drainage system, which leads to subsequent macromolecule or nanoparticle accumulation. This causes tumor-associated neovasculatures to be highly permeable, allowing the leakage of circulating macromolecules and nanoparticles into the tumor tissue. These novel properties provide exciting opportunities in developing new and advanced nanoparticle probes for biomedical imaging, especially for tumor imaging and targeting. The computational approaches will provide the analysis and interpretation of these molecular imaging data.

Nanoparticle Drugs: The use of nanoparticles for drug delivery and targeting is one of the most exciting and clinically important areas in nanotechnology. Nanotechnology is used to improve the efficacy and toxicity profiles of chemotherapeutic agents because these agents can be encapsulated, covalently attached, or adsorbed onto nanoparticles. It is also being used to overcome drug solubility problems, because more than 40% of active substances being identified through combinatorial screening programs are poorly soluble in water. Conventional and most current formulations of such drugs are frequently plagued with problems such as poor and inconsistent bioavailability. For example, Paclitaxel (Taxol) is one of

the most widely used anticancer drugs in the clinic. It is a microtubule-stabilizing agent that promotes tubulin polymerization, disrupting cell division and leading to cell death. It displays neoplastic activity against primary epithelial ovarian carcinoma, breast, colon, and lung cancers. Because it is poorly soluble in aqueous solution, the formulation available currently is Chremophor EL (polyethoxylated castor oil) and ethanol.

One of the most significant advances has been the development and FDA approval of albumin-conjugated paclitaxel (Taxol), a two-component “binary” nanoparticle (Abraxane) for treatment of taxane-refractory metastatic breast cancer. This nanoparticle formulation is shown to be effective in circumventing side effects of the highly toxic Chremophor EL such as hypersensitivity reactions, nephrotoxicity, and neurotoxicity. Recent efforts funded by the National Cancer Institute (NCI) have developed a more sophisticated “ternary” nanoparticle structure by linking both a hydrophobic cancer drug (Taxol) and a tumor-targeting ligand to a hydrophilic and biodegradable polymer. This chemical conjugation produces a graft amphiphilic polymer that is able to self-assemble into nanostructures. Computation methods systematically assess the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the drugs, and their toxicity. For example, *in vitro* cellular toxicity studies show that this new class of nanoparticle drug is similar or less toxic than free Taxol, but *in vivo* introduction to animal models bearing human tumors affected a 10-fold efficacy increase in shrinking and arresting tumors when compared to free Taxol.

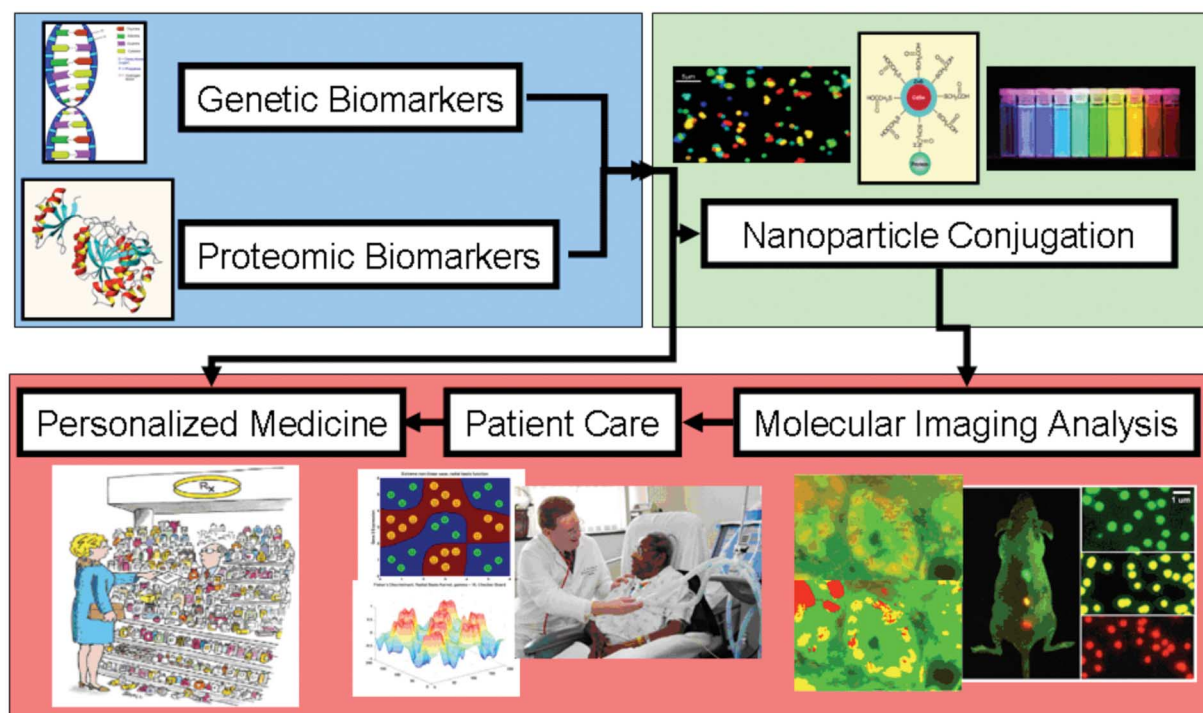
caBIG and caBIO: For personalized and predictive medicine, integrating biotechnology, nanotechnology, with information technology is critical. Personalized diagnosis, prognosis, therapeutics treatment, and eventually prevention require the knowledge of individual patient genetic make-up (see the illustration) (i.e. biomarker profiling), nanoscale probe or drug synthesis, and efficacy assessment (i.e.

imaging or drug data analysis and interpretation). A major step in integrating data and information in cancer research is the development of the Cancer Bioinformatics Grid (caBIG). It is the “World Wide Web for Cancer Research” spearheaded by the U.S. National Cancer Institute (NCI). Through data sharing and standardization, this national initiative aims to improve the infrastructures of healthcare information technology, to facilitate the process from clinical data collection to computational data mining, and to accelerate the use of biomolecular markers for personalized treatment. The caBIG organizational structure is divided into working groups, each focused on different aspects of the interoperability problem specific to cancer research. For example, work groups such as “Architecture” and “Vocabularies and Common Data Elements” produce detailed specifications to guide the design of interoperable bioinformatics tools. In practice, caBIG provides a common platform for more integrated knowledge and information. Each bioinformatics laboratory can adopt the basic infrastructure, databases, and functionality provided by caBIG working groups, and then to fill gaps in knowledge to carry out information management responsibilities. In addition to these shared tools and architectures, caBIG is a test bed for a suite of standard tools that can be understood, verified, and used by clinicians around the world. One major effort in caBIG is caBIO (Cancer Bioinformatics Infrastructure Objects), an application programming interface (API) that was first developed as Java objects and was later extended to support various programming platforms. These objects represent the most fundamental concepts in bioinformatics research such as genes, ontology, and sequences. Also under development are network programs such as caBIONet based on Microsoft's .NET technology for data analysis and integration.

Developments to Watch: The U.S. National Cancer Institute (NCI) has recently launched the Alliance for

Nanotechnology in Cancer by supporting eight national Centers of Cancer Nanotechnology Excellence (CCNE), 12 platform technology development awards, and four education and training programs (<http://www.nano.cancer.gov/>). The specific objective of this Alliance is to integrate cancer biology and nanotechnology for translating new discoveries and engineering technologies to clinical patient care. In addition, the U.S. National Heart, Lung, and Blood Institute (NHLBI) has established four centers of excellence in cardiovascular nanotechnology (<http://www.nhlbi.nih.gov/>), and the NIH Roadmap Initiative has set up a national network of eight nanomedicine development centers (<http://www.nihroadmap.nih.gov/nanomedicine/>). Looking into the future, there are a number of directions that are particularly promising, but will require concerted effort for success.

- 1) Design and development of nanoparticles with two or more functions. For cancer and other medical applications, important functions include imaging (single or dual-modality), therapy (single drug or combination of two or more drugs), and targeting (one or more ligands). With each added function, nanoparticles could be designed to have novel properties and applications. For example, binary nanoparticles with two functions could be developed for molecular imaging, targeted therapy, or for simultaneous imaging and therapy (but without targeting). Conversely, ternary nanoparticles with three functions could be designed for simultaneous imaging and therapy with targeting, targeted dual-modality imaging, or for targeted dual-drug therapy. Quaternary nanoparticles with four functions can be conceptualized in the future to have the abilities of tumor targeting, dual-drug therapy and imaging.
- 2) Nanoparticle-based molecular profiling (nanotyping) for



Bio-nano-info integration for personalized medicine.

clinical oncology and molecular pathology; that is, the use of bioconjugated nanoparticle probes to predict cancer behavior, clinical outcome, treatment response, and individualize therapy. This should start with retrospective studies of archived specimens because the patient outcome is already known for these specimens. The key hypotheses to be tested are that nanotyping a panel of tumor markers will allow more accurate correlations than single tumor markers; and that the combination of nanotyping tumor gene expression and host stroma are both important

in defining the aggressive phenotypes of cancer as well as determining the response of early stage disease to treatment (chemotherapy, radiation, or surgery).

- 3) Systematic study of nanoparticle distribution, excretion, metabolism, and pharmacokinetics in *in-vivo* animal models. These investigations will be very important in the development of nanoparticles for clinical applications in cancer imaging or therapy.
- 4) Bio-Nano-Info integration for personalized medicine including biomarker discovery/validation, multiplexed nanoimaging

data quantification, pharmacokinetics/pharmacodynamic modeling, and clinical trials design. ■

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