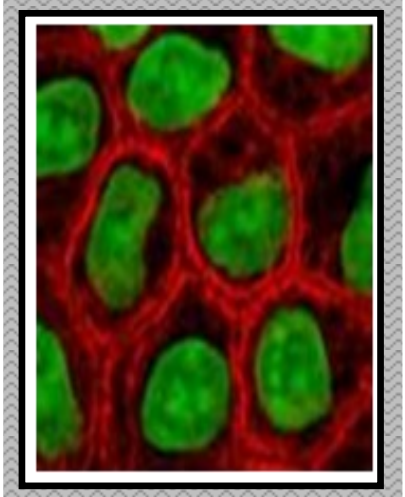

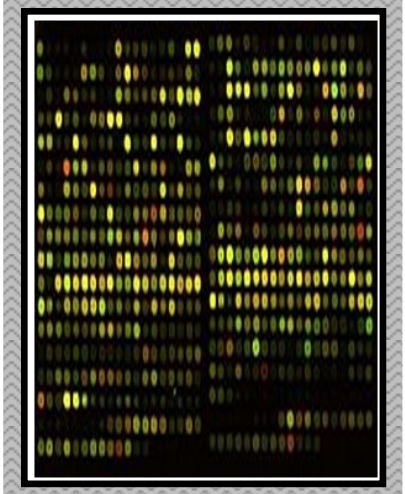

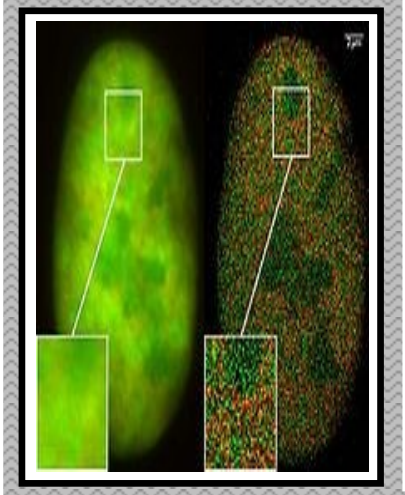

EMGEN Newsletter

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INSIDE THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMHGBN) was created in 2004 with collaboration of representatives of selected centre of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO.

Address:
Biotechnology building, #69, Pasteur Ave., Pasteur Institute of Iran
Tehran, Iran, 13164
Tel: +98-21-66954324
Fax: +98-21-66465132
E-mail: emhgbn@gmail.com, secretariat@emhgbn.net
Website: www.emgen.net

Prepared by: S. Saberi, E. Jahangeery, H. Beigi
Page Design: S. Karimzadeh
Editor: Dr. S. Sardari

Molecular characterization of *Shigella sonnei* isolates Producing Extended Spectrum β-lactamases in Lebanon

The article entitled " Molecular characterization of Shigella sonnei isolates Producing Extended Spectrum β-lactamases in Lebanon" reports molecularly characteristics of the newly emerging beta-lactam resistant Shigella sonnei, specifically ESBLs in Lebanon, and compare them to beta-lactam sensitive isolates. The study was done by Ahmad H. Sabra, George F. Araj, Mireille M. Kattar, Roland Y. Abi-Rached, Marie-Therese Khairallah, John D. Klena, and Ghassan M. Matar. Corresponding author of this paper, Dr. Ghassan M. Matar is Associate Professor of Microbiology and Immunology Department, Faculty of Medicine in American University of Beirut, and the paper was published in Journal of infection in developing countries. 2009;3(4):300-5.

Dr. Ghassan M. Matar, Ph.D.

Emergence of extended-spectrum β-lactamases (ESBLs) among members of the family Enterobacteriaceae imparting resistance to third-generation cephalosporins is a growing concern worldwide. Lately, several novel reports have indicated an increase in cases of *Shigella* species resistant to beta-lactams, including third-generation cephalosporins. So far only few cases or small case series of ESBL-producing *Shigella* have been reported from certain countries including Argentina, Hong Kong, Turkey, Korea, Bangladesh, and Palestine. Previous studies conducted in Lebanon on various species of the Enterobacteriaceae showed the prevalence of the TEM- and SHV-type ESBL-producing *E. coli* and of CTX-M-15 type ESBL.

Recently, three ESBL-producing *Shigella sonnei* isolates were isolated for the first time in Lebanon from patients with shigellosis at a tertiary medical center in Beirut.

Two additional beta-lactam resistant *S. sonnei* isolates were encountered, one of which harbored an ESBL. The aim of this study was to molecularly characterize the newly emerging β-lactam resistant *Shigella sonnei*, specifically the 4 ESBLs, in Lebanon and compare them to other collected β-lactam sensitive isolates.

Sixty *S. sonnei* isolates were collected from stool samples of different patients admitted for bacilliary dysentery to a tertiary care center in Beirut, Lebanon, between July 2004 and June 2007.

Antimicrobial susceptibility testing was performed using a panel of antimicrobials, according to CLSI guidelines 2008. Presence of ESBLs was established by the combination disk method. Eleven of the 60 isolates were selected randomly for the study and we compared five beta-lactam-resistant *S. sonnei* isolates to six isolates susceptible to beta-lactams.

These were obtained from five male and six female patients with ages ranging between 1 and 41. Total and plasmid DNA extraction, followed by PCR amplification of the three beta-lactamase-encoding gene categories (bla-CTX-M, bla-SHV and bla-TEM) in addition to the genes encoding fluoroquinolone-modifying enzymes: qnrA, qnrB, qnrS, class I and II integrons, aac (6')-Ib-cr, oxa-1, and the ISEcp1B insertion sequence were carried out. Sequence analysis of the beta-lactamase-encoding genes, along with other antimicrobial resistance genes was later performed on positive samples. The localization of beta-lactamase genes was established by conjugation experiment using the beta-lactamase positive *S. sonnei* isolates as parents and E. coli J53 (sodium azide-R) as the recipient. Plasmid-encoded blaCTX-M-15 gene was detected by PCR from both the parents and transconjugant strains. Real-time RT-PCR was used to perform relative quantification of expression of the bla-CTX-M-15 gene as compared to the housekeeping glgC gene in the presence of ceftazidime at subinhibitory concentration. Real-time PCR of the c-DNAs was performed using two sets of primers yielding a 200 bp product each targeting the bla-CTX-M-15 and the housekeeping glucose-1-phosphate adenylyltransferase (GlgC) gene. Molecular typing by pulsed-field gel electrophoresis (PFGE) was performed on the macrorestricted chromosomal DNA isolates using the PULSENET USA standard Salmonella enterica serovar Braenderup strain H9812 as a molecular size marker.

Four of five β-lactam resistant isolates were extended spectrum beta-lactamase producers. Another beta-lactam non-ESBL producing isolate was found to be resistant to ampicillin. A chromosomal bla-TEM-1 gene was detected in one beta-lactam resistant *Shigella* isolate and two of the ESBL producing isolates. PCR amplification of genomic DNA extracted from the five beta-lactam resistant *S. sonnei* isolates showed that all four ESBLs producing isolates harbored a bla-CTX-M encoding gene, while two along with one beta-lactam resistant ESBL-negative isolate, harbored a bla-TEM encoding gene. PCR amplification of the bla-CTX-M and bla-TEM genes from the plasmid DNA of the five beta-lactam resistant *S. sonnei* isolates showed the presence of only the bla-CTX-M encoding gene in all four ESBL isolates.

This indicates that the bla-TEM gene is chromosomal while the bla-CTX-M gene is plasmid-encoded. The sequence analysis demonstrated that the recovered bla-CTX-M gene in each of the four isolates was bla-CTX-M-15 and that the bla-TEM encoding gene in all three positive isolates was the bla-TEM-1 gene. None of the *S. sonnei* isolates were found to harbor a bla-SHV encoding gene or the fluoroquinolone resistance genes [qnrA, qnrB, qnrS, oxa1 or aac(6')-Ib-cr].

Conjugation experiments showed the transfer of the plasmid encoded bla-CTX-M-15 gene from the four ESBL producing *S. sonnei* isolates to the recipient J53 *E. coli*. The 4 ESBL-producing isolates were found to harbor the bla-CTX-M-15 gene and class 2 integron genes which are borne on a 70 Kb transferable plasmid. The bla-CTX-M-15 gene was flanked by an insertion element ISEcp1.Transconjugants demonstrated resistance to third-generation cephalosporins mediated by transfer of a 70 kb plasmid. The presence of a bla-CTX -M gene on the plasmid DNA of the transconjugants was determined by PCR amplification. Relative quantification ratios reflecting relative gene expression of bla-CTX-M-15/GlgC were generated by real time RT-PCR. The gene transcription levels of the bla-CTX-M-15 were increased, in EBSL isolates exposed to sub-inhibitory concentrations of ceftazidime, two- to six-fold in the tested ESBL-positive isolates. Pulsed field gel electrophoresis analysis of XbaI-restricted genomic DNA from the *S. sonnei* isolates revealed the presence of six distinct patterns that included two clusters of three and four isolates, respectively displaying identical PFGE profiles. The five bla-CTX-M-15 positive and/or bla-TEM-1 positive isolates were non-clonal. In addition, the analysis revealed two of ESBL isolates shared genotypes with β-lactam susceptible isolates indicating dissemination of resistance by horizontal plasmid transfer rather than by clonal spread of the resistant isolates.

In this study, five β-lactam resistant S. sonnei isolates and six beta-lactam sensitive *S. sonnei*, were subjected to molecular characterization. The antimicrobial resistance to third-generation cephalosporins was found to be mediated in four of five β-lactam-resistant *S. sonnei* isolates by the plasmid-borne bla-CTXM-15 gene and not by clonal dissemination, while in one of five beta-lactamase positive isolates it was mediated by the chromosomally encoded bla-TEM-1 gene only. Conjugation experiments further demonstrated that the bla-CTXM-15 gene is encoded on an approximately 70-kb transferable plasmid.

Real time RT-PCR relative quantification performed on ESBL-producing *S. sonnei* isolates demonstrated that transcription of the bla-CTX-M-15 mRNA is constitutive and independent of exposure to ceftazidime. However, the expression of this gene is further induced in the presence of ceftazidime. Macro-restriction analysis by PFGE showed that two ESBL-positive *S. sonnei* isolates had profiles identical to some β-lactam susceptible isolates. This indicates that horizontal inter- and/or within species dissemination of the 70-kb plasmid encoding the bla-CTX-M-15 gene plays a significant role in the spread of resistance to third-generation cephalosporins. Overall, there are very few band differences between the isolates on PFGE, suggesting that the *S. sonnei* isolates analyzed in this study have limited genetic diversity.

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DNA Extraction

Deoxyribose Nucleic Acid (DNA) is the master molecule, the structure which encodes all information needed to create and direct the chemical machine of life. No two people will have exactly the same DNA, except for identical twins. This makes DNA analysis a powerful identification technique. Biological data can be found in many diverse forms of samples such as liquid blood, blood or semen stains, urine, bone, teeth, hair, and skin and every living tissue. DNA material may be found on a variety of different substrates such as cloth, stone, and skin, in either a fluid or dried condition. For example, in the case of a bloodstained cloth, a cutting is made from a stained portion of the cloth and each one is placed into a tube with an extraction buffer. The tube is gently shaken and then allowed to sit for a period of time depending on the nature of the stain. The shaking tends to get rid of the biological substance for the substrate. Cellular material that has come off the sample can now be used to obtain DNA. In the cell, nearly all the DNA is found in the nucleus, which is surrounded by a host of other molecules including RNA, protein (like histone), sugars, lipids, and organic (containing carbon) and inorganic molecules. In order to analyze the nuclear DNA successfully, it must be purified and it must also be in useful form. There are several methods to remove all of these substances from the DNA. There are organic and inorganic isolation methods, for the removal of proteins and other contaminants and finally recovery of the DNA. Deletion of proteins is typically achieved by digestion with proteinase K, followed by salting-out, organic extraction, or binding of the DNA to a solid-phase support (either anion-exchange or silica technology). DNA is usually recovered by precipitation using ethanol or 70-95% isopropanol. Both procedures will produce double-strand DNA (native DNA), which is essential for RFLP analysis. Regardless of the manner in which it is isolated, the value of purified DNA may result in success or failure to obtain results.

Purified DNA should be in its native double-stranded form of high molecular weight, which means that most of the DNA should be larger than 20 kb. The reason for this is that following restriction enzyme treatment of the isolated DNA, electrophoresis of the resulting DNA fragments, Southern blotting, and fragment sizes up to 10,000 bp in length are necessary. Therefore we must detach techniques that will give high molecular weight DNA in a form valuable for various techniques. For example, successful RFLP analysis requires 50-100 ng of high-quality, nondegrated DNA. For successful PCR analysis only 1-2.5 ng of purified single-strand DNA is needed. Four steps of DNA extraction are:

1- Cell Lysis 2- Membrane Removal 3- Protein Removal 4- Recovery of DNA

Therefore, we can find different methods for extraction that some of them are explained below:

A: Salting-out Method

Cell Lysis (cell disruption): At first, the lysis solution should be added to the tube. This solution contains detergent, which breaks down cell membranes by disturbing the phospholipid bilayer, so that the DNA is released. Newly this is often done by sonication or bead beating the sample. During sonication, the particles present inside the cell are disrupted and their alignment is broken with the help of sound energy.

2- Membrane Removal: After cell lysis, the lipids present in the cells are removed by means of a detergent. SDS or chromic acid is used for washing the lipid membranes.

3- Protein Removal: The proteins (for example deoxyribonuclease), would otherwise break down the DNA, which is being isolated, may be degraded with the addition of a protease. Precipitation of the protein is aided by adding of a salt such as ammonium or sodium acetate. DNA dissolves in ionic solutions while fats, carbohydrates, and many proteins do not. Centrifugation divides the DNA from these, which sink to the base of the liquid.

4- **Recovery of DNA**: By adding ice-cold ethanol, DNA can precipitate. The DNA is unsolvable in the alcohol and will come out of solution, and the alcohol serves as a washing agent to eliminate the salt that previously added. For the reason that DNA is less dense than what stays in the solution, the DNA mass floats to the top of the alcohol layer. It shows to the naked eye similar to a clumped, string-like substance. Note: The double helix shape will not be visible—it's too small to be seen with the naked eye. The solution of 70-95% Isopropanol also is occasionally used do extract DNA precipitate. It is more efficient than ethanol, yielding a higher concentration of DNA when used.

B: Organic extraction Method

This method utilizes organic solvents to isolate contaminants from disrupted cells. The DNA is cared against unnecessary degradation in this method by adding EDTA. Proteinase K and dithiothreitol (DTT) are used for denaturation and hydrolysis of proteins. The correct salt concentration and pH must be used through isolation to make sure that contaminants are divided into the organic phase and that DNA stays in the aqueous phase. Phenol denatures proteins that are subsequently hydrolyzed. Because of their negative charge, DNA molecules can be separated from other cellular sections. During this process, the double-stranded DNA stays in the aqueous phase. DNA is usually recovered from the aqueous phase by alcohol precipitation or using a centrifugal filter unit. This is a lengthy and hard technique. The organic extraction method is used for almost all living samples except spermatozoa.

It is important to avoid introducing phenol or chloroform to the extracted DNA because it can inhibit enzyme reactions in downstream applications, and so may not be sufficiently isolated for sensitive downstream applications such as PCR.

C: DNA Extraction by Anion-exchange Method

This method utilizes the chromatography technique for isolation DNA from cells. Chromatography is based on the interaction among the negatively charged phosphates of the nucleic acid and positively charged surface molecules on the substrate. DNA combines to the substrate under low-salt situations. Cellular proteins, RNA (Ribonucleic acid) and metabolites are eliminated with the help of medium-salt buffers. The eluted DNA is picked up by alcohol precipitation, and is appropriate for all downstream applications. Anion-exchange technology entirely avoids the use of toxic substances, and is able to be used for diverse throughput requirements as well as for diverse levels of purification. The isolated DNA is sized up to 150 kb.

D: DNA Extraction Using Silica Gel

This method is simple, fast, reliable and economical for isolation of high-quality DNA. This method uses a silica-gel membrane to adsorb the nucleic acids of DNA in the presence of high concentrations of chaotropic salts that work as catalysts. The buffers used in the lysis of the cells are optimum in quantity and it assists for the adsorption of just DNA on the silica-gel membrane, while the metabolites and cellular proteins stay in solution and then are washed away. No alcohol precipitation is needed, and resuspension of the DNA, which is often difficult if the DNA has been over-dried, is not required.

E: Cesium chloride (CsCl) density gradients Method

In this method, the cells are lysed by a detergent, and the lysate is alcohol precipitated. The resuspended DNA is blended with cesium chloride and ethidium bromide, and then centrifuged for several hours. The DNA band is gathered from the centrifuge tube, isolated with 70-95% isopropanol to get rid of the ethidium bromide, and then precipitated with ethanol to pick up the DNA. This technique lets the isolation of high-quality DNA, but it is time consuming and expensive (an ultracentrifuge is essential), which making it unsuitable for usual use. This method uses toxic chemicals and is too difficult to automate.

Organic method has the least purity but the anion-exchange, CsCl and silica gel methods have the highest purity and are at the similar level. The silica gel method takes the least time, CsCl technique takes the longest time, and this method is the best but is expensive. The comparison between these techniques has been shown on a chart in figure 1.

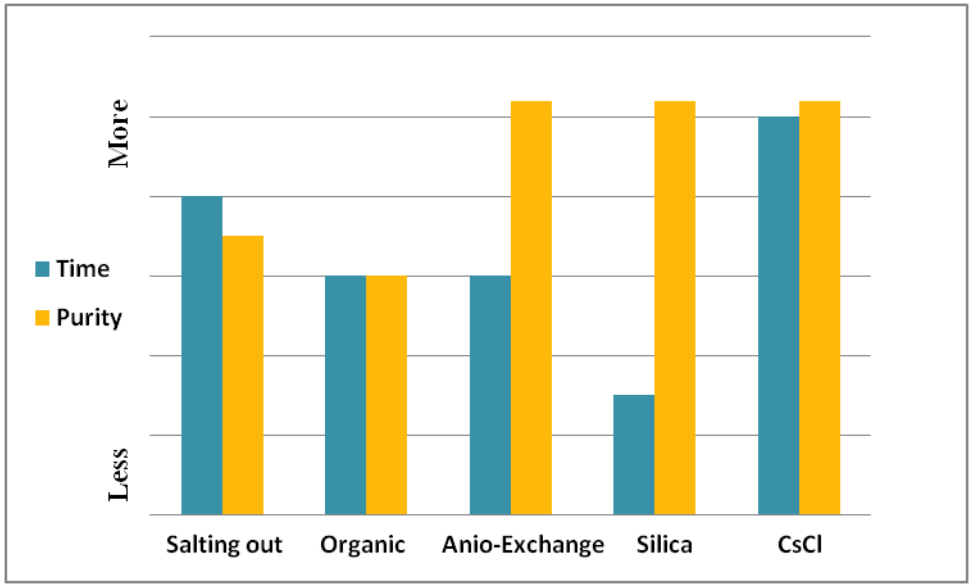

Figure1: Comparison of various DNA extraction techniques based on the resulting purity and the required time needed for the completion of the process.

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New Drug Target for Autoimmune Disease

Researchers identified a molecular target for quinoline compounds. Q compounds are used for cure of autoimmune and inflammatory diseases and are effective for multiple sclerosis (MS) and type I diabetes, Systemic Lupus Erythematosus (SLE). The researchers reported in the April 28, 2009, online edition of the journal PLoS Biology that the target was a protein called S100A9 and belongs to the family of calcium-binding S100 proteins. It is expressed in granulocytes and at early stages of monocyte differentiation. The researchers exposed that S100A9 interacted with two identified pro inflammatory receptors (Toll-like receptor 4 [TLR4] and receptor of advanced glycation end products [RAGE]), and that this interaction was inhibited by quinoline compounds. These results show that, S100A9 is a focal molecule in the control of autoimmune disease via its interactions with pro inflammatory mediators. The definite binding of quinoline-3-carboxamides to S100A9 makes clear the immunomodulatory activity of this group of compounds and defines S100A9 as a new target for cure of human autoimmune diseases.

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Advances in Synthetic Biology

4-5 March 2010 London, England

The 3rd annual Advances in Synthetic Biology conference and exhibition will take place in London in March 2010. Synthetic biology is the design and construction of new biological parts, devices and systems (and the re-design of existing, natural biological systems) for useful purposes. Its interdisciplinary nature between science and engineering, as well as the many potential applications in the health, material and energy sectors, make this a particularly interesting conference.

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a.woodley@selectbiosciences.com

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Biomarkers, bioinformatics and nanotechnology in treatment

Recent decades have witnessed an explosive growth in the amount of genomic and proteomic data, major advances in unraveling the molecular mechanisms of human diseases, and the rapid development of new technologies for molecular diagnostics and therapy. This development led to new advances in molecular medicine in which disease detection, diagnosis and treatment are tailored to each individual's molecular profile. These advances are based on the availability and application of new biomarkers for predicting disease behavior, advanced technologies for rapid detection and diagnosis, new therapies for molecular and cellular targeting and computing technologies for data analysis and management. Human diseases are often characterized by histologic lesions that are heterogeneous at the cellular and molecular levels. In cancerous tumors, for example, malignant cells are typically intermixed with blood vessels, benign stroma and inflammatory cells. Common technologies, such as gene microarrays and real-time polymerase chain reactions, are not designed to handle this type of heterogeneity, in part because they require destructive preparation of cells and tissue specimens into a homogeneous solution, leading to a loss of valuable information regarding the 3D cellular environment and tissue morphology. Recent development of nanotechnology has provided new opportunities for integrating morphological and molecular information and for correlating observed molecular and cellular changes with disease behavior.

Bioconjugated quantum dots (QDs) have been used to quantify multiple biomarkers in intact cancer cells and tissue specimens, allowing a comparative test of traditional histopathology versus molecular signatures for the same tissue. For therapy and molecular imaging, nanotechnology can be used to improve the efficacy and toxicity profiles of chemotherapeutic agents, because these agents can be encapsulated, covalently attached or adsorbed onto nanoparticles. In this study, we discuss how biocomputing and biomarkers can be integrated with nanotechnology for high-throughput analysis of gene expression data and for multiplexed molecular profiling of intact cells and tissue specimens.

Biomarkers

In medicine, a biomarker is a term often used to refer to a protein measured in blood whose concentration reflects the severity or presence of some disease state. More generally a biomarker is anything that can be used as an indicator of a particular disease state or some other biological state of an organism.

Bioinformatics tools

Bioinformatics is the application of information computer science and technology to the field of molecular biology. The term bioinformatics was used by Paulien Hogeweg in 1979 for the study of informatics processes in biotic systems. Its primary use since at least the late 1980s has been in genomics and genetics, especially in those areas of genomics involving large-scale DNA sequencing. Bioinformatics now involve the creation and advancement of algorithms, databases, computational and statistical techniques, and theory to solve problems arising from the management and analysis of biological data. Rapid developments in genomic and other molecular research technologies and developments in information technologies have combined to produce a tremendous amount of information related to molecular biology. Mapping and analyzing DNA and protein sequences, aligning different DNA and protein sequences to compare them and creating and viewing 3-D models of protein structures are Common activities in bioinformatics.

The primary goal of bioinformatics is to increase our understanding of biological processes. It's concentrate is on developing and applying computationally intensive techniques to achieve this goal. Major research efforts in the field include gene finding, sequence alignment, genome assembly, protein structure alignment, protein structure prediction, prediction of gene expression and protein-protein interactions, genome-wide association studies and the modeling of evolution.

Early in the microarray era, bioinformatics tools often focused on unsupervised clustering; moreover, the main interest was to explore new technologies and to discover new properties within the data structure without dwelling on potential clinical applications. Recent developments led to combining clustering algorithms and visualization tools into a web-based application. Numerous methods have been applied to analyzing high-throughput gene expression data from different clinical scenarios and have led to significant results concerning the identification of cancer subtypes.

Recently, the focus of analysis of microarray data started to move away from unsupervised clustering to more guided and supervised analysis. As a consequence, web-based bioinformatics applications have shifted; these newer tools focus on the analysis of genes that are differentially expressed under different known conditions.

The lists of candidate biomarkers resulting from microarray data analysis depend on both the available samples and the selection algorithm. Actually, these lists is highly unstable and often vary from sample to sample. Moreover, high-throughput assay platforms typically consist of tens of thousands of genes, that many of them are still not fully understood. Hence, the task of interpreting their results is daunting.

Each candidate gene can be associated with a biological function, one might be able to begin to understand the underlying mechanisms of the associated disease and the biological relevance of the feature selection algorithm. Databases such as the Gene Ontology (GO) database can be used to facilitate interpretation of gene functions on a large scale.

With the increasing accumulation of gene expression data, several applications have emerged with the aim of organizing and integrating the data sources and heterogeneous datasets more effectively. Increasing the data sample size can improve the reproducibility of the resulting predictive models. Thus, there has been a strong request for solutions that would allow data sharing. Array Express and Gene Expression Omnibus (GEO) are examples of large repositories that adhere to community data standards such as MIAME.

Nanotechnology and multiplexed molecular analysis

Computing tools can be used to select and optimize a small panel of biomarkers that are strong predictors for patient outcome or therapeutic response. Nanoparticles can be conjugated to antibodies and designed with the purpose of following this small set of biomarkers for molecular diagnosis and targeted therapy. Multiplexed QD probes can be used to profile a selected biomarker panel in typical clinical tissue specimens, such as tissue microarrays and needle biopsies. The use of around five to ten protein biomarkers have a significant impact on the diagnosis of a disease and the selection of individualized treatment. Xing et al. have obtained favorable results for the molecular profiling of clinical formalin-fixed paraffin-embedded (FFPE) prostate specimens. In this study, four QD–antibody conjugates have been used to recognize and detect four tumor antigens, the tumor-suppressor p53, the E3 Ubiquitin ligase murine double minute 2 (mdm-2), the zinc-finger transcription factor early growth response 1 (EGR-1) and the cyclin-dependent kinase inhibitor (CDKN) p21. These markers are important in prostate cancer diagnosis and have been correlated with tumor behavior. Recent work approved that the results from molecular profiling with QDs was consistent with results obtained by traditional immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) using human breast cancer cells. Tumor classification with the help of antigens that are expressed at low levels can be subjective and therefore requires experienced observers, and this can often contribute to considerable variations. By contrast, quantitative QD measurements are accurate and user-independent determination of tumor antigens, even when they are expressed at low levels. Consequently, the quantitative nature of QD-based molecular profiling could simplify and standardize categorization of antigens of low-abundance on intact cells and tissue specimens.

Prospects and challenges

There are several research directions that are particularly promising for biomedical applications but that require additional concerted efforts for success. The first direction of research is the design and development of nanoparticles with different functionalities. For applications in cancer and other medical conditions, relevant nanoparticle functions include imaging and therapy; nanoparticles could be developed to deliver a drug or a combination of several drugs or target one or more ligands. Via the addition of different functions, nanoparticles could be designed to have novel properties and applications. For example, binary nanoparticles with two functionalities could be used for molecular imaging and targeted therapy or for simultaneous imaging and therapy. Bioconjugated QDs with both targeting and imaging functions, could be used for targeted tumor imaging and for molecular profiling applications. Conversely, ternary nanoparticles that combine three functions could be designed so that they would allow for simultaneous imaging and targeted therapy.

The second direction is the optimization of biomarker panels via bioinformatics, quantitative molecular profiling and nanotechnology; for example, bioconjugated nanoparticle could be developed to predict cancer behavior, clinical outcome and treatment response and could help to individualize or personalize therapy. Such an approach should ideally start with retrospective studies of archived specimens because the patient outcome is known for these specimens. Here are the key hypotheses that will need to be tested: (i) a panel of tumor markers will allow more accurate correlations than single tumor markers; and (ii) the combination of molecular information of the host stroma and tumor gene expression data is necessary to define aggressive phenotypes of cancer, as well as for determining the response of early stage disease to treatment (radiation, chemotherapy or surgery).

The third research direction is to further investigate nanoparticle distribution, excretion, metabolism and pharmacodynamics in in vivo animal models. These studies will be very prominent in the development of nanoparticles for clinical applications in cancer imaging or therapy.

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http://en.wikipedia.org/wiki/Bioinformatics

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Announcement

Cover Pictures

Title: Cytokeratin
Description: Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue. The term "cytokeratin" began to be used in the late 1970s by Franke, Schmid, Osborn and Weber when the protein subunits of keratin intermediate filaments inside cells were first being identified and characterized. In 2006 a new systematic nomenclature for keratins was created and now the proteins previously called "cytokeratins" are simply called keratins.
Source:en.wikipedia.org/wiki/Cytokeratin

Title: Output from a cDNA microarray used in testing
Description: Complementary DNA (cDNA) is a single-stranded DNA that is synthesized of a single strand mRNA. It is generally formed in a laboratory by the action of the enzyme reverse transcriptase on an mRNA template. A short double-stranded sequence is required at the 3' end of the mRNA as a primer. The cDNA needs to be converted into a double-stranded DNA before it can be manipulated and cloned and it is done by DNA Pol I (Klenow fragment). CDNA is synthesized by a ribonuclease (RNase H) which distinguishes the RNA component of a DNA: RNA hybrid and cuts the RNA at a number of non-specific sites leaving short oligoribonucleotides joined to the cDNA. Complementary DNA is a popular tool for molecular hybridization studies or clone eukaryotic genes in prokaryotes.
Source: en.wikipedia.org/wiki/Complementary_DNA

Title: The green fluorescent protein (GFP)
Description: It is a protein, comprised of 238 amino acids, which its role is to transduce, the blue chemiluminescence of another protein into green fluorescent light. GFP is isolated from jellyfish, *Aequorea victoria*. GFP has been expressed in bacteria, yeast, plants, drosophila and zebrafish and in mammalian cells. The wild-type absorbance peak is at 395 nm with a minor peak at 475 nm. The availability of GFP and its derivatives has thoroughly redefined fluorescence microscopy, the GFP gene is frequently used as a reporter of expression. In modified types it has been applied to make biosensors. In this picture, a nucleus of a bone cancer cell has been shown, in left image by using usual high resolution fluorescence microscopy, it is not possible to differentiate details of its structure but in right image by using the two Color Localization Microscopy (2CLM, use GFP, RFP) it is possible to localize 70,000 histone molecules (red: RFP-H2A) and 50,000 chromatin remodeling proteins (green: GPF-Snf2H).
Source: en.wikipedia.org/wiki/Green_fluorescent_protein