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Future Drug Therapies

Abstract: Understanding drug action at the molecular level has been one of the greatest achievements of modern medicine. Characterization and classification of over eighty classes of receptors, ion channels, transporters, signal transduction enzymes and neurotransmitter synthesis/metabolic pathways enable clinicians to understand the molecular mechanisms of the drugs they use in the practice of medicine. Radiolabelled ligands are available for all the targets of drug action enabling researchers to screen new compounds in search of better drugs. Complete pathway diagrams are available for about 20 signaling pathways which provide clues for potential targets for new drug development. Nanotechnology has the power to radically change the way cancer is diagnosed, imaged and treated. Nanoscale devices such as dendrimers can be specifically targeted to cancer cells delivering therapeutic agents within the target cells or even within specific organelles, while totally avoiding normal cells.

Pharmacogenetics and pharmacogenomics are going to play an increasingly important role in the development of better medicines for the population and targeted therapies with better benefit/risk ratio for the individual patient. Nutrigenomics studies the interaction between nutrition and genes. Many dietary phytochemicals possess anti-inflammatory, antimutagenic and anticarcinogenic properties via suppression of TNF- α induced Koch's to gene transcription, and NF κ B and AT1 activation and induction of apoptosis. Chemoprevention of atherosclerosis, neurodegeneration and cancer by dietary phytochemicals holds the promise for the future. Molecular nanotechnology (MNT) or nanorobotics will allow *in vivo* molecular surgery on individual cancer cells. Using microelectromechanical systems (MEMS) and nanoelectromechanical system (NEMS), nanomedical devices can be created as exemplified by "Microbiovore," an artificial white blood cell, and "Respirocyte," an artificial red blood cell.

INTRODUCTION

Understanding drug action at the molecular level has been one of the greatest achievements of modern medical science. Application of techniques such as X-ray, crystallography and H^1C^{13} NMR spectroscopy has provided information enabling the characterization and classification of over eighty classes of receptors, ion channels, transporters, signal transduction enzymes and neurotransmitter synthesis/metabolism pathways that will enable clinicians to understand the molecular mechanisms of action of the drugs in current practice. Radiolabelled ligands are now available for all the above targets of drug action, enabling researchers to screen new compounds in search of better drugs.¹

CELL SIGNALING PATHWAYS (Fig. 16.1)

We are witnessing a virtual explosion of knowledge about cell signaling pathways impacting virtually all areas of molecular biology and medicine. Complete pathway diagrams are available for about 20 signaling pathways (such as MAPK/ERK in growth and differentiation).

Protein kinases constitute one of the largest human gene families and are key regulators of cell function, especially those that carry signals from the cell membrane to intracellular targets

and coordinate complex biological functions. The 518 human protein kinases control protein activity by catalyzing the addition of a negatively charged phosphate group to other protein. Over 200 phosphospecific antibodies are now available for assessing the expression of these kinases in tissue (immunohistochemistry), in lymphocytes, in peripheral blood (flow cytometry) or on cell membranes (immunofluorescence or Western blotting).

The 20 signaling pathways (Table 16.1) provide clues about potential targets for new drug development in various disorders— cardiovascular, metabolic, inflammatory, degenerative or neoplastic.

ANTI-CANCER DRUGS

Greater understanding of cellular and molecular biology of normal cell growth and proliferation provides potentially important new targets for drug design. In particular, there is considerable interest in intracellular signaling pathways which modulate the effects of peptide growth factors operating by autocrine mechanisms in transformed cells. Growth factor receptor blockade, inhibition of oncogene expression, synthetic “non-functional” analogues of the endogenous intermediates in the signal transduction cascade, or direct modulators of activities of key enzymes such as protein tyrosine kinases, are some of the new approaches. One needs a multiprong approach to block the key intracellular regulators of cellular transformation, inducing apoptosis of the tumour cells and concurrently inhibiting neovascularization in tumour cells while sparing the normal cells.

Several polypeptide hormones and growth factors have been identified which contribute to neoplastic transformation and they are over-expressed on cancer cells, e.g. Vasoactive intestinal peptide (VIP), somatostatin (SST) bombesin and substance P. DRF 7295 a combination of 4 synthetic neuropeptide analogues can selectively block adenocarcinomas both *in vitro* and *in vivo*. It has pro-apoptotic, cytotoxic and antiangiogenesis effects compared to fluorouracil (5FU), irinotecan and oxaliplatin plus leucovorin. A combination may improve outcome in colorectal and breast cancer.

TARGETED THERAPY ANTI-ANGIOGENESIS

The role of angiogenesis in growth, progression and metastasis of solid tumours has been known for decades. Hematological malignancies (myeloma, leukemia) are associated with increased angiogenesis in the bone marrow. Prognosis of myeloma depends on the degree of marrow microvascular density. Thalidomide which was withdrawn in the 1960s due to its teratogenic effects, has made a re-entry due to its anti-angiogenesis properties. Its usefulness was proved in refractory and relapsed cases of multiple myeloma during the last five years, so that hematologists are considering the question: Why not *start* with thalidomide? (Rajkumar 2003)² Lenalidomide, an analog of thalidomide is 300 times more potent, with different side effect profile (no constipation, sedation or neuropathy) but greater myelosuppression.

Another novel anti-angiogenesis approach is the use of a synthetic analogue of $\alpha\gamma\beta3$ integrin (highly expressed in angiogenesis) complexed to cationic nanoparticles to deliver a therapeutic gene to tumor-associated vascular endothelial cells 4.³

UBIQUITIN PROTEASOME PATHWAY

Dr Aaron Ciechanover from Israel received the Nobel Prize in Chemistry for the year 2004 for his work on the Ubiquitin Proteasome Pathway. Proteasome is a multi-catalytic complex, responsible for degradation of ubiquitinated intracellular proteins. Activation of NF KB occurs in the proteasome, from its binding to IKB. A new drug Bortezomib (Velcade) blocks the proteasome from breaking IKB-NFKB to its active form, thereby depriving the growth stimulus to myeloma

cells. Today, Velcade has emerged as the most powerful approach for treating multiple myeloma both for new as well as relapsed patients.

NANOMEDICINE AND CANCER

Nanotechnology has the power to radically change the way cancer is diagnosed, imaged and treated. There are ongoing efforts to design novel nanodevices capable of detecting cancer at its earliest stage, pinpointing its location within the body and delivering anticancer drugs specifically to malignant cells, totally avoiding normal cells.

Nanoscale devices smaller than 50 nanometers can easily enter most cells, while those smaller than 200 nanometers can transit out of blood vessels. Hence, they can interact with biomolecules on both the cell surface and within the cytoplasm delivering therapeutic agents within the target cell or even within specific organelles. Despite its small size a nanoscale device is capable of holding tens of thousands of small molecules such as drugs or contrast imaging agents. Even toxic drugs can be delivered at much smaller doses in a controlled and time release manner.

Nanoscale delivery devices such as *dendrimers* linked to specific antibodies against tumor associated surface ligands can specifically target cancer cells. Folate nanoparticles have higher specific affinity for human cancer cells hence, they improve the uptake of the encapsulated drug. Nanocarriers overcome the problems of poor bioavailability, short half life, insolubility and instability and undesirable side effects and toxic effects of anti-cancer drugs. Nanocarriers penetrate better the leaky tumor cell membrane with 100-1000 nanometer sized pores.

NANOSHELL-ASSOCIATED PHOTOTHERMAL THERAPY (NAPT)

Nanoshells have a core of silica coated with an ultrathin metallic layer, usually gold. By adjusting the core and shell thickness, nanoshells can be designed to absorb and scatter light at a desired wave length. Using nanoshells that absorb light in the near infrared (NIR) region and then convert it into heat (40°C), and delivering them specifically to cancer cells via enhanced permeation retention (EPR), selective photothermal ablation can be achieved in a non-invasive way using a laser in the NIR region.

Nanoshells also have an interesting application in the treatment of insulin-dependent diabetes mellitus. Instead of several daily injections of insulin, the patient will use a ball point pen size infrared laser to heat the skin wherein a nanoshell polymer containing insulin has been injected. The heat from the nanoshell will cause the polymer to release a pulse of insulin. The nanoshell polymer system could remain in the body for months. Nanospectra Bioscience (www.nanospectra.com) hope to start clinical trials for this insulin delivery system by end of 2006.

MOLECULAR NANOTECHNOLOGY (MNT)

Molecular nanotechnology (MNT) or nanorobotics will allow performance of direct *in vivo* molecular surgery on individual human cells. The ability to design, construct and deploy large numbers of microscopic medical nanorobots will make this possible.⁴

It is an interesting thought that the human body is full of micro-robots in the form of red blood cells, leucocytes (neutrophils, monocytes/ macrophages, lymphocytes) and fibroblasts, that are constantly moving inside our bodies, repairing damaged tissue, attacking invading microbes, gathering foreign particles and transporting them to various organs for disposal from the body. Hence, it is possible to develop prototype microscale assemblers using micro-electromechanical systems (MEMS) and nano-electromechanical systems (NEMS). Two interesting examples are: "Microbiovore" an artificial white blood cell, and "respirocyte" an artificial red blood cell.

Microbiovore, an artificial white blood cell is an oblate spheroidal nanomedical device measuring 3.4 μ diameter along its long axis and 2 μ diameter along its short axis, consisting of 610 billion precisely arranged structural atoms in a gross geometric volume of 12 μ^3 and a dry mass of 12.2 picograms. It is 80 times more efficient in phagocytosis than macrophages, in terms of

volume/second digested per unit volume of phagocytic agent. During each cycle of operation the target bacterium is bound to the surface of the microbiovor in the blood stream like a fly on fly paper via species-specific binding sites, and transported to the ingestion port. After sufficient mechanical mincing it goes to a digestion chamber wherein a pre-programmed sequence of 40 engineered enzymes are successively injected and extracted six times progressively reducing the morcellate ultimately to monoresidue aminoacids, mononucleotides, glycerol, free fatty acids and simple sugars. These simple molecules are then harmlessly discharged back into the blood stream through an exhaust port, completing the 30 second cycle. Microbiovors can clear the most severe septicemia within a few hours or less time compared to weeks by the natural mechanism.

Respirocytes

Robert Freitas has designed an artificial mechanical “respirocyte” which is a one micron size diamondoid 1000 atmospheric pressure vessel with active pumping powered by endogenous serum glucose. It is able to deliver 236 times more oxygen to the tissue per unit volume than natural RBC and to manage carbonic acidity. Gas concentration sensors on the outside of each device let the nanorobot know when it is time to load O₂ and unload CO₂ (at the lung) or *vice versa* (at the tissues). Injection of a 5 ml therapeutic dose of 50% respirocyte-saline suspension delivers 5 trillion respirocytes that can replace the gas carrying capacity of the patient’s entire 5 litres of blood.

PHARMACOGENETICS AND PHARMACOGENOMICS

Pharmacogenetics and pharmacogenomics are expected to play an important role in the development of better medicines for the population and targeted therapies with improved benefit/risk ratios for the individuals with their unique biology. The genetic and genomic information coded in disease pathophysiology or treatment response directs drug development and changes medical practice through identification of new molecular targets, measurement of biological effects, understanding causation of outcomes, prediction of prognosis and response (or lack of it) or toxicity of the therapy.^{5,6}

It is now possible to test the phenotype of patients with MDR genotype causing rapid clearance of drugs like indinavir, ritonavir and 3 TC, which helps the clinician to choose other drugs like AZT, Viread and lamivudine.

Selecting the best drugs for the individual patient is now possible, through pharmacogenetic information, e.g. CYP2C9 testing before warfarin, TPMT testing before 6 MP and AZA, etc.⁶

CARDIOVASCULAR DISEASE

Genomics, in combination with transcriptomics, proteomics and metabolomics, will help pharmacogenomics in predicting individual responses to drugs as well as adverse drug reactions. The identification of biomarkers of human cardiovascular disease has been attempted using microarrays and cardiochip which contains about 10,000 genes, including all genes isolated from the cardiovascular system, in order to illustrate how gene expression can affect myocardial damage. 200-250 genes are involved in heart failure.

A multinational project GEMS (Genetic Epidemiology of Metabolic Syndrome) aims at a phenotype identification and the susceptibility genes with a micro-array for 20,000 genes it is now possible to study gene expressions during exposure of human endothelial cells to hypoxia.

A total of 17 classes of drugs are used to treat cardiovascular disorders. A comprehensive pharmacogenomics approach is necessary for their optimal usage. For example, SNPs of HMGCoA reductase influence LDL response to statins, which determine the magnitude of response to statin, in different ethnic populations.⁷

OATP-C, the organic anion transporting polypeptide C is responsible for hepatocellular uptake of statins. SNPs in the OATP-C coding region, are responsible for interindividual variability in response to statins.

NUTRIGENOMICS

The field of studying how nutrition and genes interact is called nutrigenomics. Apart from heritable food allergies, dietary factors can modulate serum HDL levels. Significant interaction is observed between the intake of polyunsaturated fatty acids, the apolipoprotein genes APOA-1 and APOA5 and lipoprotein remnants, as well as between intake of fat, variability at the hepatic lipase locus and HDL-C. The effects of maternal malnutrition on the fetus, and the suppression of IRS-1-P13K pathways leading to insulin resistance and metabolic syndrome are well known.

Just as many dietary constituents can increase the risk of developing atherosclerosis and cancer, dietary phytochemicals possess substantial anti-inflammatory, anticarcinogenic and antimutagenic properties. The intracellular signalling cascades have been identified as common molecular targets for various chemopreventive phytochemicals, e.g. NF κ B and AP1, MAPK/ERK, and AKT signaling pathways. Curcumin (active principle of Haldi) suppresses TNF α induced COX2 gene transcription and NF κ B and AP1 activation, and induces apoptosis. Similar effects are seen with capsaicin (chillies), caffeic acid (Honey), Diallyl sulphide (garlic), epigallocatechin EGCG (green tea), Gingerol (ginger), Genistein (soyabean), indole3-carbinol (cabbage), lycopene (tomatoes), resveratrol (grapes), sulphoraphane (broccoli).⁸

FUTURE MEDICAL PRACTICE

The future scenario of medical practice is going to be a change for the better. Instead of empirically prescribing a "standard treatment," the doctor will order a genetic profiling that will help determine the right treatment for the particular patient. A further development will be for healthy individuals to determine their disease susceptibility genetic profile so that a 20 year window of opportunity is provided for preventing the expression of these genes through alteration of the environment such as avoidance of smoking, alcohol, pollution, lifestyle modification including diet, (low fat and high intake of fruits and vegetables) exercise and behaviour. This change has to take place now if we hope to prevent being overwhelmed by the burden of obesity, hypertension, diabetes, degenerative disorders and cancer 25 years from now. Chemoprevention of atherosclerosis, neurodegeneration and cancer with phytochemicals holds the promise for the future.

REFERENCES

1. The Sigma-RBI Hand Book of Receptor Classification and Signal Transduction (4th edn). 2001 Ed. KJ. Watting Sigma-RBI Natick MA, USA.
2. Rajkumar SV. Why not start with thalidomide? J Clin Onco 2003;21:2212-4.
3. Robert A. Frietas Jr. Current status of nanomedicine and medical nanorobotics J Computa and Theoretical Nanoscience 2005;2:1-15.
4. Robert A Frietas Jr. Editorial nanotechnology nanomedicine and nanosurgery. Int Jour Surgery 2005.
5. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: Current status and future prospects. FASEBJ 2005;19:311-30.
6. Lele RD. The Human Genome Project: Its Implication in Clinical Medicine.
7. Willard HF, Angrist M, Ginsburg GS. Genomic medicine: Genetic variation and its impact on the future of health care. Phil Trans R Soc B 2005;360: 1543-50.
8. Young-Joon Surh. Cancer chemoprevention with dietary phytochemicals: Nature Reviews 2003; 3(10):768-80.