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MOLECULAR MEDICINE 2005:
A CHALLENGE FOR THE FUTURE

THE ROLE OF RNA PROCESSING DEFECTS IN HUMAN DISEASE. DIAGNOSTIC AND THERAPEUTIC_IMPLICATIONS.

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Human Genetic Diseases are caused by variations in the genomic sequences that affect one of the fundamental steps of gene expression. In this presentation particular attention will be given to the pre mRNA splicing process. This a complex mechanism that follows the first step of expression, the transcription of DNA into RNA and involves the correct identification of protein coding sequences (exons) from the more abundant non coding sequences (introns). When sequence variants are identified in genomic DNA, especially in disease-associated genes, the correct interpretation of the molecular nature of the substitution may not be immediately evident. The effect of the mutations on gene expression is generally defined according to their location. Missense and non sense exonic genomic variations affect the coding potential and are always considered as pathogenic. Silent mutations (ie that do not change the amino acid coding capacity) are routinely disregarded as potential cause of disease. Intronic mutations, if not at the splice sites, are largely ignored. However in many cases these straightforward conclusions are wrong and the primary mechanisms of disease are catastrophic splicing abnormalities that result from the disruption of unrecognized splicing regulatory elements. That this represents a not uncommon possibility comes from the recent estimate that the cause of 15 % of all genetic diseases concerns dysfunction in the pre-mRNA splicing process. In addition, the distinction between benign polymorphisms and splicing pathogenic mutations is an increasing diagnostic challenge. We will describe examples of genetic disease arising from splicing defects in several regulatory elements and their diagnostic and therapeutic implications for diseases such as: Cystic Fibrosis (CFTR), Ataxia Telangiectasia (ATM), Homocistinuria (CBS), and Neurofibromatosis (NF1).

These studies have also intriguing evolutionary implications. In fact it is usually assumed that the driving force of evolution is improvement of protein function. However as there is a substantial overlap between the RNA sequences coding for a protein and splicing regulatory elements, it follows that at times optimal protein function may have to be sacrificed to allow exon inclusion in the mature mRNA.

This issue together with the splicing factors and supramolecular complexes involved correct and defective pre mRNA splicing will be discussed together with the relevant techniques used for their analysis and the potential therapeutic approaches that can be explored in the light of new knowledge of the RNA processing mechanisms.

MOLECULAR MECHANISMS OF BILIRUBIN TOXICITY: OLD CONCEPTS REVISITED

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Unconjugated bilirubin (UCB), the major degradation product of heme, was long considered to be simply a toxic metabolite that must be cleared from the organism. In the past two decades, it has become appreciated that UCB is a «boon» at physiological or mildly elevated concentrations, due to its potent protective action against oxidant cell damage, but may be a «curse» at more elevated, yet still clinically-relevant concentrations, due to its cytotoxic effects, especially in the central nervous system (CNS)[1, 2]. The moderate «physiologic» jaundice that develops after birth has been suggested to be neuroprotective for the neonate, due to the antioxidant properties

of unconjugated bilirubin (UCB)[3]. By contrast, if the underlying immaturity of the hepatic transport processes and/or the post-natal increases in production and enterohepatic circulation of UCB are more severe, marked neonatal jaundice occurs that may result in reversible neurotoxicity (bilirubin encephalopathy)[4]. This may progress to precipitation of UCB in focal areas of the central nervous system (CNS) with permanent neurological damage (kernicterus)[5].

The effects of UCB on disease incidence and prognosis have been only recently acknowledged and may be related to its known effects on cellular function, mostly determined from *in vitro* studies. They vary from the potent antioxidant effect at low concentrations but may be prooxidant at higher concentrations[6, 7]) to the limitation of the increases of Tumor Necrosis Factor-α, Nitric Oxide and inducible NO Synthase by indirect activation of the Constitutive Androstane Receptor (CAR) or direct binding to and activation of the Aryl Hydrocarbon Receptor (AhR)[8]. Bilirubin also inhibits Vascular Cell Adhesion Molecule (VCAM1)-dependent migration of leukocytes[9].

Collectively these considerations suggest that the plasma (and, therefore, tissue) levels of UCB might affect the incidence and or/severity of a variety of diseases, not only those related to the increased level of the pigment in newborns. At low, physiological levels, UCB is a highly potent antioxidant that apparently protects individuals against common diseases that are related to oxidant stress. This is supported by a strong negative correlation between serum bilirubin levels and several non hepatic diseases such as atherosclerosis (in particular coronary artery disease), cancers, demyelinating neuropathies and seasonal affective disorder. An exception is schizophrenia, which is more frequent and severe when the plasma UCB is increased[10].

The evidence that high-normal to modestly elevated plasma bilirubin levels might a boon for the body have several implications. First the protective range of plasma UCB levels should be established and utilized in assessing risks of specific cardiovascular, oncological and neurological diseases. This addition in the evaluation of risk may have important effect particularly on the population based screening. In parallel, prospective studies should be initiated to chronically raise or lower plasma bilirubin levels, particularly in high-risk subjects, to determine whether this is beneficial in disease prevention. Data are needed also regarding the biological effects of bilirubin at various Bf levels and the mechanisms of these effects.

Clearly unconjugated bilirubin, until recently regarded as a waste product of the heme metabolism, must be considered to be an active molecule with many functions and therapeutic potential, yet to be explored.

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MOLECULAR IMAGING AS A KEY COMPONENT OF THE WORLD OF MOLECULAR MEDICINE: EVOLUTION OF DIAGNOSTIC IMAGING: FROM TRADITIONAL TO MOLECULAR IMAGING

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Molecular medicine is transforming discoveries derived from the study of human genome and proteomics into clinically useful interventions to improve the diagnosis, treatment and management of disease. The understanding of the mechanism of disease at the cellular level is accelerating the development of new molecular medicines. Molecular imaging agents, an important and growing sector of molecular medicine, offer the potential to

address unmet clinical needs through molecular imaging and targeted radiotherapeutics[1]. The term molecular imaging can be broadly defined as the *in vivo* characterization and measurement of biological processes at the cellular and molecular level[2]. Contrary to the «classical» diagnostic imaging, this new approach is targeted to probe the molecular abnormalities that are the basis of the disease, rather than imaging the end-effects of these molecular alterations. Since most of agents currently used in clinical setting are unspecific, they do not highlight specific disease but rather unspecific pathological tissue changes. Molecular imaging combines new molecular agents with traditional imaging tools to create targeted, tailored therapies with the ability to simultaneously find, diagnose and treat disease. Currently being investigated for numerous applications, including oncology, cardiology and neurology, molecular imaging will offer significant benefits over standard diagnostics and treatments[3,4]. Molecular imaging consists in the characterization and assessment of the biologic processes at the molecular level in a non invasive way, and may reveal the difference in the molecular structure which differentiate «healthy» from «sick» tissue[5]. Only recently we start developing molecular *probes* and associated imaging technologies that are highly specific for detecting *in vivo* predetermined molecular targets in a three dimensional fashion. A molecular probe must have two important characteristics: 1) high affinity for interacting to a target molecule (e.g. a protein) associated with a specific type of disease, e.g., breast and prostate cancer; and 2) easy labeling with a marker molecule that can be tracked by a suitable detector placed outside the body[2,3]. The label could be a radioactive tracer for NM, a paramagnetic agent for MRI, micro-bubbles for US and an optically absorbing dye for optical imaging. To date, NM**,** comprising positron emission tomography (PET) and single photon emission tomography (SPECT), plays a major role in molecular imaging. These techniques possess excellent sensitivity, whole-body applications, good reproducibility and quantitation due to the use of radiolabeled molecules that allow to image molecular interactions of biological processes in vivo[6,7]. However, their poor spatial resolution make them unsuitable for image-guided drug delivery, and require relatively long scan times. Higher spatial resolution has been obtained by developing hybrid systems with x-ray-based imaging such as PET-CT. Magnetic Resonance is having an important role in molecular imaging based on its good spatial resolution and applicability to all body regions. Highly sensitive contrast agents possibly allowing imaging of molecular targets and gene expression are under development taking benefit from the experience in the design of molecular probes for NM[5,9]. Ultrasound and optical imaging, relatively new imaging techniques, will also use molecular probes and take advantage of their safety and invasiveness to assess physiological information and be used for image-guided drug delivery and enhancement of (gene-) therapy[9].

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