08 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

What is epigenetics?

459
What is Epigenetics?

M. Esteller[1]
[1]*Centro Nacional de Investigaciones Oncológicas (CNIO), Cancer Epigenetics Laboratory, 3rd Floor, Molecular Pathology Program, Madrid, Spain*

An altered pattern of epigenetic modifications is central to many common human diseases, including cancer. Many studies have explored the mosaic patterns of DNA methylation and histone modifications in cancer cells on a gene-by-gene basis, among them the seminal finding of transcriptional silencing of tumor suppressor genes by CpG island promoter hypermethylation. Epigenetic gene inactivation in transformed cells involves many "belts of silencing". We are in the process of completing the molecular dissection of the entire epigenetic machinery involved in methylation-associated silencing, such as DNA methyltransferases, methyl-CpG binding domain proteins, histone deacetylases, histone methyltransferases, histone demethylases and Polycomb proteins. The first indications are also starting to emerge about how the combination of cellular selection and targeted pathways leads to abnormal DNA methylation. In addition to classical tumor-suppressor and DNA repair genes, epigenetic gene silencing includes genes involved in premature aging and microRNAs with growth inhibitory functions. Recent technological advances are now enabling cancer epigenetics to be studied genome-wide. It is time to "upgrade" cancer epigenetics research and put together an ambitious plan to tackle the many unanswered questions in this field using genomics approaches to unravel the epigenome.

08 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Promises, challenges and pitfalls in large data analysis

460
Biomarkers and surrogate endpoints in clinical research - some statistical challenges

M. Buyse[1]
[1]*IDDI, Biostatistics, Ottignies Louvain-la-Neuve, Belgium*

Recent developments in biostatistics and bioinformatics are reshaping clinical research. With the number of promising new molecules available for clinical testing, clinical trials need to detect a drug's benefit (and harm) as fast as possible. In parallel with the need for speed in clinical development, advances in molecular biology, high throughput technologies and imaging techniques provide investigators with an ever growing number of biomarkers which can be used for a variety of purposes: to inform go / nogo decisions in early clinical development, to stratify patients, to target subsets, to adjust treatments, or to replace / support clinical endpoints for drug approval. This talk will briefly cover all of these goals, and will discuss the level of evidence required for a biomarker to be useful in every case. The talk will mostly focus on the use of biomarkers as surrogates for clinical endpoints. It will be shown that two criteria need to be fulfilled before a biomarker can be considered a valid surrogate for a clinical endpoint: there must be a strong "individual-level" association between the biomarker and the clinical endpoint, and also a strong "trial-level" association between the effects of a treatment (or class of treatments) on the biomarker and the clinical endpoint.(1) The latter criterion is seldom looked at, and is (surprisingly) not implied by the former. Showing that both criteria are met usually requires a meta-analysis of randomized trials. When such data are available, the predictive value of potential surrogate biomarkers can be investigated, and the "surrogate threshold effect" can be estimated as the minimum effect on the surrogate biomarker that predicts a statistically significant effect on the clinical endpoint. (2) The talk will use actual datasets in oncology to illustrate all of these notions.

References:
1. Buyse M., Molenberghs G., Burzykowski T., Renard D., Geys H. The Validation of surrogate endpoints in meta-analyses of randomized experiments. Biostatistics 1: 49-68, 2000
2. Burzykowski T., Buyse M. Surrogate threshold effect: An alternative measure for meta-analytic surrogate endpoint validation. Pharmaceutical Statist 5: 173-186, 2006

08 July 2008 09:00 - 11:00

SYMPOSIUM

New targets

461
BRAF and RAS signalling in human melanoma

R. Marais[1]
[1]*Cancer Research UK Centre for Cell and Molecular Biology, The Institute of Cancer Research, London, United Kingdom*

The RAS-RAF-MEK-ERK signalling pathway is a critical player in human melanoma. This pathway is hyper-activated in the majority of human melanomas, largely because NRAS is mutated in ~15% of melanomas and BRAF is mutated in another 50-70% of cases. Signalling through this pathway stimulates proliferation and survival of melanoma cells and the pathway has been established as an important therapeutic target in this disease. We have developing mouse models of melanoma driven by oncogenic BRAF or oncogenic RAS. We find that inducible expression of oncogenic BRAF from the endogenous mouse gene stimulates a progressive disease that is characterized by the appearance of naevi followed by the induction of melanoma 6-12 months after the expression of oncogenic BRAF in the melanocytic lineage. Importantly, the tumours arise in the mice that have not been manipulated in any other manner and which have a wild-type genome. These data indicate that oncogenic BRAF is a powerful inducer of melanoma in mice. However, the long delay required for the appearance of the lesions indicates that oncogenic BRAF alone is not sufficient for complete progression from melanocyte to melanoma and that additional genetic events are required. We have also shown that expression of oncogenic KRAS in the melanocyte lineage leads to melanoma. Thus, we have developed a model of melanoma that are driven by oncogenic BRAF and oncogenic KRAS. The tumours appear to possess many of the features found in the human disease and these mice will therefore be powerful tools for the ongoing genetic analysis of melanoma.

462
PI3-kinases and cancer

G. Mills[1]
[1]*University of Texas, Department of Molecular Therapeutics, Houston Texas, USA*

The phosphatidylinositol-3-kinase (PI3K) pathway plays a crucial role in cell growth and survival and is activated in cancer. Multiple components of the pathway are frequently targeted by amplification, mutation and translocation in cancer patients. Indeed, the breadth and frequency of genomic aberrations in the PI3K pathway in cancer patients indicates a critical role in tumor initiation and progression and further validate the pathway for targeted therapeutics. The frequency of aberrations in the PI3K pathway exceed that of any other pathway with the possible exception of the p53 pathway. However, crosstalk with the p53 and retinoblastoma pathways comprises a signalling network that promotes tumour initiation and progression. Further as the PI3K pathway is activated in tumors and consists of multiple kinases, it is a target rich environment. Despite major interest in this pathway for drug discovery efforts against cancer, no drugs have yet been approved that act specifically against PI3K or the downstream regulator, Akt. However, several drugs that were developed for other purposes either directly or indirectly target PI3K signaling, such as the rapamycin analogs, ether lipids such as perifosine and miltefosine, and inhibitors of the epidermal growth factor receptor (EGFR), HER2, c-kit, platelet-derived growth factor receptor and bcr–abl. Because of the crucial role of the PI3K pathway in normal cell growth and in response to stress, the main challenge to developing PI3K drugs is to identify inhibitors with a usable therapeutic index. Tumors with aberrations in the PI3K pathway may undergo "oncogene addiction" rendering them sensitive to inhibition of the PI3K pathway providing a potential therapeutic index. It is likely that PI3K inhibitors will need to be used in combination with other drugs that cause cell stress, such as other signaling inhibitors, radio- and chemotherapy. Points at which therapeutic intervention might be appropriate in the PI3K

pathway include targeting PI3K itself, the downstream regulator Akt, although inhibiting this crucial signaling node might result in toxicity, and other downstream components such as mTOR, integrin-linked kinase (ILK), phosphoinositide-dependent kinase-1 (PDK-1), p70S6 kinase and Forkhead/FOXO1. As with other molecularly targeted agents including imatinib mesylate (Gleevec) and trastuzumab (Herceptin), the success of PI3K inhibitor drugs will likely depend on the selection of cancer patients likely to be responders and non-responders based on genomic aberrations. The co-development of molecular markers determine early responders allowing triage to effective will increase utility of the targeted agents.

463
Choline kinase is a novel prognostic marker and a therapeutic target in human cancer

J.C. Lacal[1]
[1]*Centro Nacional de Biotecnología, Translational Oncology Unit CSIC-La Paz-UAM, Madrid, Spain*

Cancer cells have altered signalling pathways that are usually associated to the loss of their ability to differentiate, become blind to apoptosis-triggering signals, and are hyper-reactive or autonomous to proliferation signals. However, in order to proliferate, they still need to replicate DNA and some key cellular components such as membranes. Furthermore, cancer cells have altered key metabolic pathways that may become very fragile. By contrast, the plasticity of normal cells is based on solid grounds where metabolic pathways are quite robust and efficient. A proper understanding of the most critical signalling and metabolic pathways required to maintain cancer cells, will facilitate the design of very specific strategies that may specifically affect cancer cells with very little effect on normal cells. One of such approaches will be discussed on the light of the metabolic control on one of the potential Achilles heels of cancer cells: the pathways that control phospholipids metabolism. Regardless of the specific signals that promote cell cycle entrance and DNA replication, all cancer cells require the simultaneous increase in phospholipids synthesis. Furthermore, a link between this pathway and the generation of potent toxic metabolites exists. Thus, this metabolic pathway becomes an interesting mechanism to control cancer cells viability. As a model for this novel antitumoral approach, the design of specific inhibitors to choline kinase, one of the enzymes involved in phosphatidylcholine synthesis, will be reported. The antitumoral effect of such compounds is fully supported by the understanding of their mechanism of action. The enzymatic system that regulates phosphatidylcholine synthesis is more complex that anticipated. Recent progress in this field will be discussed.

464
The TGF-beta pathway in cancer

J. Seoane[1]
[1]*Vall d'Hebron Universtity Hospital Research Institute, Medical Oncology, Barcelona, Spain*

The oncogenic effect of the TGF-beta pathway has prompted the design of several compounds to be used as anti-TGF-beta therapies in cancer. It is crucial to understand the molecular pathways implicated in the malignant role of TGF-beta in oncogenesis in order to select the patient population that may benefit from an anti-TGF-beta therapy. We have focused our studies on the oncogenic role of TGF-beta in glioma. In some glioma tumours, TGF-beta acts as an oncogenic factor. We have demonstrated that high TGF-beta-Smad activity is present in aggressive, highly proliferative gliomas and confers poor prognosis in patients with glioma and we have discerned the mechanisms and molecular determinants of the TGF-beta oncogenic response using a transcriptomic approach and analyzing human glioma biopsies, primary cultured patient-derived tumour cells, and patient-derived glioma stem cells. We have observed that TGF-beta exerts its proliferative function through the induction of PDGF-B. Moreover, we have found that human glioma stem cell self renewal is regulated by TGF-beta. Glioma stem cells are considered to be responsible for glioma initiation, maintenance and recurrence, and hence are optimal therapeutic targets against this deadly disease. We have identified the molecular mechanisms that regulate the self-renewal capacity of glioma stem cells through TGF-beta.

08 July 2008 09:00 - 11:00

SYMPOSIUM

Role of microRNAs in cancer

465
Functional genetic approaches identify cancerous miRNAs

R. Agami[1], C. Calos le Sage[1], R. Remco Nagel[1]
[1]*The Netherlands Cancer Institute, Department of Tumor Biology, Amsterdam, The Netherlands; Reuven Agami Department of Tumor Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, 1066CX*

microRNAs (miRNAs) are potent post-transcriptional regulators of protein coding genes. Patterns of mis-expression of miRNAs in cancer suggest key functions of miRNAs in tumorigenesis. However, current bioinformatics tools do not fully support the identification and characterization of the mode of action of such miRNAs. To perform genetic screens for novel functions of miRNAs we developed a library of vectors expressing the majority of cloned human miRNAs and created corresponding DNA barcode arrays. In a screen for miRNAs that cooperate with oncogenes in cellular transformation we identified miR-372 and miR-373, each permitting proliferation and tumorigenesis of primary human cells that harbor both oncogenic RAS and active wild type p53. We provide evidence that these miRNAs are potential novel oncogenes participating in the development of human testicular germ cell tumors by numbing the p53 pathway, thus allowing tumorigenic growth in the presence of wild type p53. Recently, we have used a novel functional genetic approach and identified miR-221 and miR-222 (miR-221&222) as potent regulators of p27Kip1, a cell cycle inhibitor and tumor suppressor. Interestingly, high miR-221&222 levels appear in signatures of poor prognosis cancers. Using miRNA-inhibitors we demonstrated that certain cancer cell lines require high activity of miR-221&222 for the maintenance of low p27Kip1 levels and continuous proliferation. Thus, high levels of miR-221&222 promote cancerous growth by inhibiting the expression of p27Kip1. Last, we performed experiments to uncover metastasis promoting miRNAs. We describe the role of miR-373 in cellular migration and metastasis of breast cancers. Thus, we find functional genetic experiments extremely useful in the identification and characterization of cancerous miRNAs.

466
Small RNAs in animal development

R.F. Ketting[1], S. Houwing[1], L. Kamminga[1], H. Huang[1]
[1]*Hubrecht Institute (KNAW), RNA Silencing, Utrecht, The Netherlands*

Piwi interacting RNAs (piRNAs) are a class of small RNAs that in many species is abundantly expressed in the germline. We and others have shown that their biogenesis differs significantly from mi- and siRNAs in at least two steps. First, piRNAs carry a chemical modification at their 3' most terminal nucleotide. This modification, an O-methyl on the 2'OH group, is deposited by a homologue of the plant Hen1 protein. Second, piRNAs are not made by Dicer. Instead, strong evidence has been obtained in Drosophila that piRNAs are made through Piwi protein mediated cleavages, where one type of Piwi protein generates the 5' ends of a new piRNA that will be loaded into a different Piwi paralogue. This model has been named the ping-pong model and piRNA sequences from fish and mice display signatures that are consistent with it. We have analyzed piRNAs binding to both Piwi proteins, both in testis and ovary. A clear ping-pong signature emerges, with Ziwi binding to antisense piRNAs and Zili to sense. In addition, we find both Piwi proteins to interact, and have identified a number of other Zili interactors. Furthermore, Piwi protein localization is very dynamic, ranging from diffuse cytoplasmic localization to granular staining along the cell and/or nuclear membrane, and complete intra-nuclear localization at specific stages of germ cell development. These findings indicate that Piwi proteins in zebrafish likely affect both cytoplasmic, such as transposon mRNAs, and nuclear targets, for example chromatin. Nuclear effects of Piwi proteins are further supported by an oocyte specific block in meiosis displayed by a zili hypomorphic allele. Finally, we will present data from C. elegans that hint at an age-dependent effect of RNAi on the fidelity of meiosis and chromosome segregation during the first cell divisions.